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POSTER ABSTRACT BOOKLET/ PROGRAMME & RÉSUMÉS

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CSM Canadian Society of Microbiologists
SCM Société canadienne des microbiologistes

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GENERAL INFORMATION/ INFORMATIONS GÉNÉRALES

This booklet summarizes the submitted abstracts received by attendees at this year's CSM-SCM 2026 University of Alberta conference, held June 16-19, 2026. These abstracts include those scheduled for poster sessions, abstracts selected for invited talks, and for workshops. For a detailed list of the keynote and invited symposium session speakers, please refer to the CSM 2026 website:
https://event.fourwaves.com/csm2026/schedule

The names and email addresses of all presenting abstract authors and conference registrants can be found in the **Author Index**, for anyone interested in reaching out to presenters about their work.

Instructions for those presenting a poster (or presenting in the various competitions) are also included in this booklet (please refer to the Table of Contents). All poster presentations this year are held as in-person sessions. Our virtual program, only includes online access to the keynote and award lectures (Murray, Fisher, Armand Frappier), Student and Postdoctoral Symposium competitions, and selected thematic symposia as marked as "Hybrid" in the online schedule. On Friday, June 19, the three CSM Section Symposia will also be available for virtual viewing, through a Zoom link available on the schedule (see the link above).

The CSM Executive Committee and Local Organizing Committee at the University of Alberta wish you all to have a wonderful and engaging experience at this year's conference.

Ce livret regroupe les résumés soumis par les participants au congrès CSM-SCM 2026 de UAlberta (16 au 19 juin 2026). Ces résumés comprennent ceux prévus pour les séances d'affiches, les présentations invitées et les différents ateliers. Pour obtenir la liste détaillée des conférenciers principaux et invités, veuillez consulter le site web du congrès CSM-SCM 2026:
https://event.fourwaves.com/csm2026/schedule

Les noms et adresses courriel de tous les auteurs des résumés présentés sont disponibles dans l'index des auteurs, pour toute personne souhaitant communiquer avec les présentateurs au sujet de leurs travaux.

Les instructions pour les personnes présentant une affiche (ou participant aux différents concours) sont également incluses dans ce livret (veuillez consulter la table des matières). Cette année, toutes les présentations par affiche se déroulent en présentiel ; notre programme virtuel comprend uniquement l'accès en ligne aux conférences principales, à la conférence de remise des prix, aux conférences (Murray, Fisher, Armand Frappier), aux concours oraux pour étudiants et postdoctorants, et à certains symposiums thématiques, indiqués par la mention « Hybride » dans le programme en ligne. Le vendredi 19 juin, les trois symposiums des sections de la SCM seront également accessibles en ligne, soit via le lien Zoom, soit en direct, là où la mention « Hybride » est indiquée dans le programme en ligne.

Le comité exécutif de la SCM et le comité organisateur local de l'Université de Alberta, à Edmonton (Alberta), vous souhaitent la bienvenue et se réjouissent de vous accueillir à la conférence de cette année.

Dr. Tracy Ravio, Ph.D.	**Valeria Tsviklist**	**Stephanie Carter**
CSM LOC Chair and organizer	LOC Co-organizer	LOC Co-organizer

PDF SYMPOSIUM COMPETITION/ CONCOURS DU SYMPOSIUM PDF

This section provides highlights of the general abstracts submitted by this year's six top-ranked Post-doctoral fellows (PDF) competing in the PDF Symposium based on their extended abstract submissions by the CSM Meeting Secretary and the CSM Section Chairs. PDFs selected for this competition are not required to present their abstract as a poster during the General poster session.

Cette section présente les points saillants des résumés généraux soumis par les six postdoctorants les mieux classés de cette année, sur la base des résumés détaillés soumis par le secrétaire du congrès et les présidents des sections du CSM. Les postdoctorants sélectionnés pour ce concours ne sont pas tenus de présenter leur résumé sous forme d'affiche lors de la session générale d'affiches.

P1 - Role of colonic MUC2 mucin and pro-inflammatory cytokines in innate host defence against adherent-invasive Escherichia coli infection



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Inflammatory bowel disease (IBD) includes two chronic diseases, Crohn's disease and ulcerative colitis of the gastrointestinal tract associated with complex interactions among host genetics, immune responses, environmental factors, and the intestinal microbiota. Dysbiosis is a hallmark of IBD and is characterized by altered microbial composition and reduced diversity. Among bacterial taxa enriched in IBD patients, adherent-invasive *Escherichia coli* (AIEC) has emerged as important pathobionts capable of adhering to and invading intestinal epithelial cells and promoting intestinal inflammation. In a healthy gut, the intestinal epithelium is protected by a thick viscous mucus barrier produced by goblet cells composed of gel-forming MUC2 mucin, which spatially separates luminal microbes from the epithelial surface. At present, it is unclear how AIEC interacts with the mucus barrier to gain access to epithelial cells in disease pathogenesis.

To investigate the role of the mucus barrier in host protection against AIEC, we used complementary ***in vitro*** and ***in vivo*** models of the disease. AIEC adherence and invasion assays using wild-type (WT) and *MUC2* deficient (*MUC2* KO) LS174T goblet-like cells revealed significantly higher intracellular AIEC invasion in *MUC2* KO cells, indicating that the mucus layer restricts bacterial invasion. Confocal microscopy confirmed temporal intracellular invasion, with greater bacterial entry in *MUC2* KO cells. In contrast, non-pathogenic *E. coli* DH5α did not invade either cell type. Exposure of purified MUC2 mucin to AIEC secreted enzymes, degraded both the glycosylated and C-terminal regions of MUC2. Consistent with increased bacterial invasion, infection induced time-dependent expression of the pro-inflammatory cytokines (*IL-8*, *TNF-α*, and *IL-1β*), with significantly higher responses in *MUC2* KO cells.

To assess the role of the mucus barrier *in vivo*, $Muc2^{+/+}$ and $Muc2^{-/-}$ littermates were infected with AIEC NRG857c. While both genotypes initially showed high levels of bacterial colonization, WT mice progressively cleared the infection, whereas $Muc2^{-/-}$ littermates maintained high bacterial burdens throughout the 20-day period, demonstrating impaired clearance in the absence of Muc2. In WT littermates, AIEC infection induced increased *Muc2* expression and elevated Muc2 protein temporally. These findings were corroborated using *Atoh1*-reporter mice as a proxy for goblet cell lineage activity, by excised colon imaging, that showed a transient increase in goblet cell differentiation during infection. Taken together, these findings demonstrate that the colonic mucus barrier plays a critical protective role in limiting AIEC invasion and persistence. While, AIEC can overcome this defence through mucin degradation and epithelial invasion, the host responds by enhancing mucus production and goblet cell activity to restore barrier integrity and to elicit pro-inflammatory cytokines in innate host defence.

Section Type

I&I

P2 - Viral Biogeography in the Healthy Human Body

Amanda Araujo Serrao de Andrade[1, 2], Andre Ontal[1], Andrea Silverj[1, 2], Isadora Bischeck do Nascimento[1], Ann Gregory[1, 2]

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Viruses play a fundamental yet understudied role in shaping human health beyond their traditional association with disease. Although research has largely focused on pathogenic viruses, most of the human virome comprises bacteriophages, which shape microbial communities and can modulate immune responses. These viruses can transcytose across the gut epithelia and spread throughout the human body. Understanding the distributions of viruses across the human body and their interactions with different cell types across tissues and systems is key to assessing their impact on human health and their potential as therapeutics. Here, we characterized the distribution of viruses across healthy organs by compiling large-scale sequencing datasets, assessing viral presence in classically sterile organs, and constructing a map of viral-human cell interactions.

The study encompasses 202.07 terabytes of public RNA-seq and single-cell RNA-seq data from 28 independent projects, comprising 11,874 samples spanning brain, heart, lung, spleen, pancreas, lymph node, bone marrow, thymus, liver, and kidney. All reads were first aligned to the masked human genome (hg19), the ERCC database, UniVec, and the phage phiX174 using Bowtie2. Unmapped reads were assembled and screened with DeepVirFinder, Vibrant, VirSorter2, and GeNomad. Similarity-based searches were then performed against the masked databases ViralRefSeq, Unified Human Gastrointestinal Genome, BacteriaRefSeq, ArchaeaRefSeq, and FungiRefSeq. To identify low-complexity sequences, we used RepeatMasker with RepBase and Dfam databases. The remaining contigs were searched using tBLASTx against all six reading frames of the masked human genome (hg19). An additional CheckV analysis was performed to determine prophage coordinates and assign a confidence level to the viral origin of each contig. This analysis resulted in the identification of a total of 606,304 viral sequences with N50 = 1,419 base pairs (bp). Viral sequences were filtered into two subsets: contigs >1,500 bp (n = 132,991; N50 = 2,706bp) and contigs >5,000 bp (n = 7,625; N50 = 6,165bp), to target ssRNA, ssDNA, and dsDNA viruses. All others were excluded as low-confidence. After this filtering, we have identified viral sequences in all analyzed organs, including classically sterile sites. The number of contigs varied by organ, with liver showing the highest counts (46,237, N50 = 2,435bp), followed by heart (46,062, N50 = 2,924bp), brain (13,588, N50 = 4,569bp), and kidney (14,936, N50 = 2,570bp). While most viral sequences were unclassifiable, those that could be assigned were primarily Caudoviricetes (tailed phages), of which 12.1% were integrated prophages. In the pancreas, spleen, and lung, 81.1% of viral sequences were associated with immune and endothelial cell types, consistent with viral uptake by host cells or transcytosis mechanisms. Host prediction indicated 52% of viral sequences likely infect gut-associated bacteria, including *Bacteroides* and *Parabacteroides*. Regarding functional annotation, viral sequences encoding marker genes such as RNA-dependent RNA polymerase, DNA polymerase, tail fiber protein, portal protein, terminase large subunit, and major capsid protein were identified. These findings reveal unexpected viral diversity in classically sterile human tissues and underscore the importance of characterizing gut viral transcytosis and viral-human cell interactions in healthy individuals.

Section Type: MGCM

P3 - Therapeutic candidate phage KS12 selects for antimicrobial hypersensitivity and attenuated virulence through increased outer membrane permeability.



Philip Lauman[1,2], Nora Hussain[1], James Stafford[1], Jonathan Dennis[1]

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The widespread overuse of antibiotics has predictably driven the emergence of resistance, leading to eroded antimicrobial efficacy, a rise in untreatable infections, and increased interest in alternative antimicrobial strategies. Key among these approaches is phage therapy, which utilizes bactericidal viruses (bacteriophages) to selectively infect and destroy bacterial pathogens. In particular, recent work has focused on antivirulence phage steering, a strategy wherein phage predation is leveraged as a selective pressure to bias bacterial populations toward more clinically tractable phenotypes. In parallel, increasing attention has been directed toward the immunological consequences of phage exposure and whether phages stimulate host responses that influence therapeutic outcomes.

Burkholderia gladioli is an opportunistic pathogen that causes severe pneumonia and septicemia in individuals with cystic fibrosis and other immunocompromising conditions. *B. gladioli* is intrinsically resistant to many antimicrobials, however, and infection with this pathogen is considered a contraindication to lung transplantation, leaving affected patients with few therapeutic options. Although *B. gladioli* therefore represents a compelling target for phage therapy, bacteriophages infecting this species remain largely uncharacterized and no studies have systematically examined the antibacterial efficacy, antivirulence steering capability, or immunogenicity of *B. gladioli* phages. Here, we evaluated the therapeutic and immunogenic potential of lytic *Burkholderia* myovirus KS12 and explored the capacity of this phage to steer bacterial populations toward clinically tractable phenotypes.

KS12 effectively suppresses *B. gladioli* growth across diverse *in vitro* conditions and significantly reduces infection-associated mortality *in vivo*, supporting the therapeutic potential of this phage. Although KS12 is readily inactivated by humoral innate immunity, it does not independently stimulate pro-inflammatory immune responses and appears to interact minimally with professional phagocytes, indicating that the therapeutic activity of this phage arises primarily from direct bactericidal activity rather than activation of the innate immune system.

As an LPS-binding phage, KS12 selects for resistant subpopulations carrying O-antigen biosynthesis and export pathway mutations that compromise outer membrane integrity, resulting in attenuated virulence *in vivo* and hypersensitivity to human serum, antimicrobial peptides, and polymyxins. Consistent with this trade-off, KS12 and colistin exhibit order-dependent antibacterial synergism that decimates populations of *B. gladioli*, suggesting that predation by KS12 may impose an evolutionary trap in which escape from infection increases susceptibility to treatment with antibiotics. Moreover, comparative analyses indicate that outer membrane permeability correlates strongly with colistin susceptibility across several Gram-negative pathogens, implying that antivirulence steering with LPS-binding, OM-destabilizing phages could serve as a general strategy to sensitize pathogens to antibiotics of last resort.

Together, our findings identify the lytic, non-immunogenic myovirus KS12 as a promising therapeutic candidate against *B. gladioli* and highlight the potential of LPS-targeting phages to act not only as antibacterial agents, but also as drivers of antivirulence steering. This approach enables evolutionary reprogramming of intrinsically resistant pathogens toward attenuated virulence and increased susceptibility to immune effectors and antimicrobials, resulting in more clinically tractable phenotypes.

Section Type

I&I

P4 - Microbiology of a membrane aerated biofilm reactor upgrade for a municipal wastewater treatment facility

Michelle McKnight[1], Ahmed Yousif Abdalla[1], Narasimman Lakshminarasimman[1], Jose Bicudo[1], Wayne Parker[1], Josh Neufeld[1]

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The membrane aerated biofilm reactor (MABR) is a novel wastewater treatment biotechnology that is considered attractive because of its promise to reduce energy consumption and improve nitrification and nitrogen removal under cold weather conditions. A municipal wastewater treatment plant (WWTP) in southern Ontario was upgraded with an MABR system that was installed upstream of the existing conventional activated sludge (CAS) system. Here we evaluated spatial and temporal impacts of the MABR upgrade on the mixed liquor and MABR biofilm microbial communities. Microbial communities were characterized using 16S rRNA gene amplicon sequencing, with selected MABR biofilm samples analyzed with metagenomics to evaluate the functional potential of the biofilm for nitrification and denitrification. Before the upgrade, the CAS mixed liquor included the nitrifiers *Nitrosomonas* (ammonia-oxidizing bacteria; AOB) and *Nitrotoga* (nitrite-oxidizing bacteria; NOB), which exhibited seasonal abundance and activity patterns. After the upgrade, seeding from the MABR biofilm increased diversity of the mixed liquor, including nitrifiers. Along with AOB, *Nitrospira* NOB and complete-ammonia oxidizing (comammox) *Nitrospira* were detected in MABR biofilms. Metagenomic functional profiling predicted that biofilm microbial communities were equipped to perform nitrification and denitrification in the system, in addition to phosphate removal. Overall, characterization of microbial communities in the WWTP showed that the MABR installation increased microbial diversity, concomitant with increased representation of nitrifier groups. Recent characterization of the MABR system after two years of operation, revealed stable microbial communities within MABR biofilm. Consistent nitrifier populations were found for MABR system biofilm samples and were further increased in diversity compared to initial post upgrade profiles, including the appearance of previously undetected *Nitrosocosmicus* populations (ammonia-oxidizing archaea; AOA). Overall, increased nitrifier diversity was consistent with improved nitrogen removal observed in the plant. Continued monitoring of the system is underway to further assess microbial community composition of the MABR system under seasonal cycles and assess long-term stability of microbial communities, with a focus on microbial nitrogen removal capacity.

Section Type

AEM

P5 - Discovery of putative active and cryptic viral infections in prevalent gut fungi



Andrea Silverj[1, 2], Amanda Araújo Serrão de Andrade[1, 2], Kyla Ost[3], Ann Gregory[1, 2]

[1]Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada, [2]Calvin, Phoebe, and Joan Snyder Institute for Chronic Diseases, University of Calgary, Calgary, Alberta, Canada, [3]Department of Immunology and Microbiology, University of Colorado Anschutz School of Medicine, Aurora, Colorado, USA

Fungi represent a critically understudied component of the human gut microbiome, yet they play important roles in host health and disease. Among fungal pathogens, *Candida* species are responsible for the majority of human fungal infections, contributing to nearly one million deaths per year worldwide. This burden is worsened by the growing association between *Candida* infections and chronic illness, immunosuppression, and the emergence of antifungal-resistant strains, underscoring the urgent need for novel preventive and therapeutic strategies. Viruses that infect fungi, known as mycoviruses, represent a largely unexplored way for controlling fungal growth and virulence. While metagenomic studies have catalogued hundreds of thousands of viral species in the human gut, most characterized viruses are bacterial. Only a small fraction of mycoviruses have been described, and those associated with *Candida* remain poorly understood. This knowledge gap is partly due to a historical bias in metagenomics toward DNA viruses, whereas most known mycoviruses have RNA genomes.

To address this gap, we conducted a large-scale exploration of the *Candida* virome by analyzing all publicly available metatranscriptomic data across ten *Candida*-like species, a diverse group collectively responsible for most human fungal infections. We retrieved 6,175 RNA-seq samples from the NCBI Sequence Read Archive spanning ten health-relevant *Candida* species. To further maximize mycovirus recovery, we also generated 43 new RNA-seq samples using an optimized protocol that avoids poly-A enrichment. All datasets underwent quality control, and host *Candida* transcripts were removed prior to assembly with rnaSPAdes. Assembled contigs were screened through a comprehensive viral identification pipeline incorporating state-of-the-art tools and databases, with phylogenetic analyses performed under a maximum-likelihood framework to reconstruct the evolutionary history of identified viruses.

Our preliminary screening identified 1,919 putative viral contigs, of which 223 showed relatedness to previously characterized fungal viruses. Viruses were detected in seven of the ten *Candida* species examined. These putative mycoviruses spanned 13 viral orders and 16 viral families, with RNA virus families predominating, including *Narnaviridae*, *Mitoviridae*, *Partitiviridae*, *Hypoviridae*, *Orthototiviridae*, and others. Notably, the *Genomoviridae* family, characterized by a single-stranded DNA genome, was also detected, with transcriptomic evidence suggesting active replication within the fungal host. Viral diversity varied considerably across species: *Candida albicans* and *Candidozyma auris* harbored viruses from multiple families, while *Candida parapsilosis*, *Debaryomyces hansenii*, and *Pichia kudriavzevii* were associated exclusively with *Orthototiviridae*. Importantly, several viruses were identified in type strains widely used in laboratory research, highlighting that unrecognized viral infections may confound experimental outcomes in fungal biology. Differential expression analyses to characterize virus-host interactions are currently ongoing.

This work represents, to the best of our knowledge, the most comprehensive survey of the *Candida* virome to date, significantly advancing our understanding of fungal virus diversity and laying the groundwork for the development of mycovirus-based antifungal therapeutics.

Section Type

AEM

P6 - Persistent Oral Candida Infections: Clinical Presentation and Antifungal Resistance in Older Northern Ontario Adults



Karolina Czajka[1, 2], Yamamah Al-Jumaili[1, 2], Krishnan Venkataraman[1, 2], Danielle Brabant-Kirwan[3], Stacey Santi[3], Chris Verschoor[1, 2, 3], Vasu Appanna[1], Ravi Singh[2, 3], Deborah Saunders[2, 3], Sujeenthar Tharmalingam[1, 2, 3]

[1]Laurentian University, [2]NOSM University, [3]Health Sciences North Research Institute

Oral candidiasis is a common fungal infection caused by microbial dysbiosis that disproportionately affects older adults and immunosuppressed individuals. Despite its prevalence, diagnosis and treatment remain challenging due to diverse clinical features and potential for antifungal resistance. Clinicians must currently assess each case individually, with signs and symptoms such as xerostomia, burning mouth, angular cheilitis, removable plaques, and oral redness to guide treatment decisions. Selecting the correct antifungal is complicated by antifungal-resistant species which cannot be visually distinguished apart from susceptible strains.

The main objective of this study was to determine what oral candidiasis signs and symptoms serve as indicators for *Candida* infections especially oral antifungal-resistant ones. Additionally, the impact of medical conditions and frailty were assessed in terms of oral candidiasis risk and successful treatment. Last, antifungal resistant strains were characterized with culturing methods and molecular biology techniques like RNA sequencing and RT-qPCR.

57 patients aged 65 years and older (mean age 74) and most actively receiving cancer treatment (65%), were enrolled through oncology clinics in Northern Ontario. The medical history of each patient was complex, and no significant factor predicted a resistant infection, including frailty with 45% of patients classified as moderately to severely frail. Most infections involved *Candida albicans (*61%) and responded to standard antifungal treatment (69%). In contrast, infections involving *Nakaseomyces glabratus* and *Pichia kudriavzevii* (with known antifungal resistance) tended to persist (83% and 100% respectively). Symptomatically, pseudomembranous candidiasis characterized by white plaques, coated tongue, and taste disturbance, was more likely to resolve, while erythematous features such as angular cheilitis and oral redness were associated with persistent infection. Characterization of antifungal-resistant *C. albicans* and *N. glabratus* strains demonstrated upregulation of resistance mechanism associated genes *ERG11* and *CDR1.*

These findings underline the clinical variability of oral candidiasis and highlight the need for rapid molecular diagnostic tools at the point of care to distinguish infection types and guide appropriate therapy. This is especially important for at-risk populations in Northern Ontario, where access to specialized oral healthcare is limited and there is a need for improved approaches to oral health assessment. Additionally, key findings demonstrate the need to refine our understanding of fungal load thresholds and to investigate how fungal-bacterial interactions within the oral microbiome contribute to oral health, frailty, and systemic health outcomes in aging populations.

Section Type

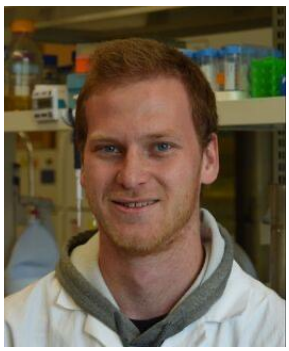
MGCM

STUDENT SYMPOSIUM COMPETITION/ CONCOURS DE SYMPOSIUM ÉTUDIANT

This section provides a listing of the general abstracts submitted by this year's twelve top-ranked student symposium extended abstract submissions by the CSM Education and Career Committee and CSM Section Chairs. Students selected for this competition are not required to present a poster.

Cette section présente la liste des résumés généraux soumis par le Comité de formation et de carrière et les présidents de section du SCM parmi les douze meilleurs résumés soumis pour la compétition "*symposium des étudiants*" de cette année. Les étudiants sélectionnés pour ce concours ne sont pas tenus de présenter une affiche.

S1 - Systematic screening of reporter-based mutant libraries reveals the lack of a dominant pathway controlling biofilm matrix production in Staphylococcus aureus



Jean-Sébastien Bourassa[1], Elizabeth Gaudreau[1], Jean-Philippe Côté[1], Pascale B. Beauregard[1]

[1]Université de Sherbrooke

Antimicrobial resistance continues to rise globally, with well-established multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) remaining a serious public health threat. In Canada alone, MRSA causes nearly 10,000 infections and 400 deaths annually. The epidemic USA300 lineage of *S. aureus* now accounts for most of community-associated MRSA infections, surpassing healthcare-associated cases in both Canada and the United States. USA300 is a leading cause of skin and soft-tissue infections and invasive infections.

A key determinant of *S. aureus* pathogenicity is its ability to form biofilms, which promote persistence and antimicrobial resistance. However, comprehensive genetic analyses of biofilm formation have been hampered by limited genetic tools and labor-intensive assays such as crystal violet staining.

To address this, we developed a high-throughput approach that combines dynamic luminescent bioreporters targeting two major components of the USA300 biofilm matrix, i.e., adhesins (P_{fnbA}) and extracellular DNA (P_{cidA}), with a large mutant library. Each reporter was introduced into 1919 of the 1920 Nebraska Transposon Mutant Library mutants via high-efficiency transduction, thereby generating two new reporter libraries. Reporter activity was monitored during biofilm growth and served as a proxy for matrix production in each strain.

We identified 71 and 77 mutants affecting P_{fnbA} and P_{cidA} expression, respectively. Gene ontology analysis revealed that defects in energy production pathways led to increased *fnbA* expression, consistent with small-colony variant phenotypes. In contrast, mutations that reduce P_{cidA} activity highlighted flavin and riboflavin metabolism, suggesting a potential role for these pathways in programmed cell death and biofilm formation. Notably, only six mutants affected both reporters similarly, indicating distinct regulatory mechanisms for both matrix components.

Among key candidates, *mntR* and *yjbH* emerged as regulatory crossroads. YjbH modulates the stability of the stress and biofilm regulator Spx, explaining its phenotype. In contrast, the role of MntR in biofilm regulation remains to be clarified. Both mutants displayed altered fibronectin-binding and biofilm formation, supporting the functional relevance of our approach.

Collectively, our results prove the robustness and extensibility of this approach. Using our optimized transduction protocol, a complete reporter-tagged mutant library can be generated in less than two weeks. Although our initial screening was performed in liquid culture, adaptation to solid media formats would enable higher-density screening (up to 1536 mutants per plate), further accelerating genetic analyses.

In summary, this strategy, which combines sensitive luminescent reporters with large-scale mutant libraries, dramatically accelerates screening and allows precise monitoring of complex bacterial phenotypes. By overcoming major limitations in high-throughput phenotyping, our approach provides a powerful tool for dissecting staphylococcal infection mechanisms and facilitating the discovery of new targets to combat biofilm-associated infections. Future work will focus on incorporating this system into more clinically relevant models.

Section Type

MGCM

S2 - The regulation of biofilm formation in a Crohn's disease associated Escherichia coli strain

Jonas Wong[1, 2, 3, 4], Ahmad Refaat[1, 2, 3, 4], Pablo Villacampa-Teixeira[5, 6], Jonasz Patkowski[5, 6], Natalie Lapa[1, 2], Julián Ortiz[1, 2, 4], Kasia Dzierlega[1, 2], Blake Roberts[1, 2], Stephanie Tollenaar[7], Matthew Croxen[2, 8], Aducio Thiesen[9], Dina Kao[10], Xavier Clemente-Casares[1, 2], Ben Willing[7], Tiago Costa[5, 6], Wael Elhenawy[1, 2, 3, 4, 11]

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Bacterial type IV secretion systems (T4SSs) are dynamic molecular machines involved in processes ranging from DNA exchange to biofilm biogenesis, providing multifaceted functionality for strains carrying them. Their role in biofilm development has emerged as a key factor in the persistence of adherent-invasive *Escherichia coli* (AIEC), which are frequently isolated from Crohn's disease (CD) patients where they aggravate gut inflammation (Elhenawy et al., *Nat. commun.,* 2021). Many AIEC strains carry IncF-like plasmids encoding expanded T4SSs adapted for conjugation and pilus elaboration, and T4SS-dependent biofilm formation is essential for AIEC fitness in the gut. Here, we describe a unique AIEC strain carrying an IncF plasmid with a noncanonical T4SS architecture that lacks two essential core components: TraC, a VirB4-like ATPase that energizes pilin polymerization, and TraV, a lipoprotein required for outer membrane core complex assembly (Wong et al., *Nat. commun.,* 2025). These missing components are supplied by homologues encoded on a chromosomally integrated conjugative element (ICE), giving rise to a hybrid secretion system. Regulation of the two systems is independent, where IncF *tra* genes are repressed by the FinO/FinP axis under standard conditions, while ICE gene expression is governed separately. Derepressing IncF genes alone was insufficient to enhance biofilm formation or pilus elaboration; only upon co-expression of ICE-encoded TraC and TraV did biofilm formation increase ~2.4-fold. Direct visualization confirmed that co-expression of both ICE-encoded components increased the piliated bacterial population, establishing these components as rate-limiting for hybrid T4SS assembly in a derepressed IncF background. Despite their independent regulation, both the IncF and ICE clusters are coordinately induced by live intestinal epithelial cell contact *in vitro*, as well as *in vivo* in a murine infection model. These findings uncover a previously unrecognized regulatory cooperation between plasmid- and chromosomally encoded T4SS components, revealing how two independently regulated mobile genetic elements are coordinately induced by host cues to drive biofilm formation and persistence in CD-associated *E. coli*.

Section Type

MGCM

S3 - Comparing and Characterizing the Microbiomes of Industrialized Populations and the Yanomami Indigenous Population from the Remote Rainforests of Brazil and Venezuela



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Microbial ecology in the human gut is driven by intricate interactions among species—cross-feeding, cooperation, and competition—that determine the stability and resilience of communities. However, the mechanisms underlying these interactions remain poorly understood, in part because most studied systems are highly disturbed by modern environments that obscure ancestral ecological states. In contrast, populations living in minimally industrialized conditions preserve microbial lineages and metabolic capacities that more closely reflect natural, undisturbed metabolic networks and community dynamics. The functional repertoire of non-industrialized gut microbial populations is far more varied as they contain diverse and greater abundances of short-chain fatty acid (SCFA) production genes and vitamin biosynthetic pathways. These are crucial for facilitating microbe-microbe interactions and cross-feeding networks resulting in deep richness and diverse microbial compositions.

Industrial gut microbiotas have demonstrated shifts in key functionality such as an increased ability to degrade simple carbohydrates, host glycans, and sugar substitutes reflective of modern diets. As a result, microbial diversity in these ecosystems have decreased resulting in functional losses and taxonomic deficits. The group of microbes primarily found in non-industrial populations but rarely in industrialized populations carry out unique functions and are classified as VANISH (Volatile and/or Associated Negatively with Industrialized Societies of Humans) However, microbiomes of industrialized societies have gained microbes that carry functions adjusted to modern lifestyles and classified as BloSSUM (Bloom or Selected in Societies of Urbanization/Modernization). To date, no study has investigated whether there are microbial taxa that persist in both groups despite their diverse lifestyle differences. This study leverages a vast range of human fecal metagenomic datasets to investigate how conserved taxa endure across ecological gradients and how microbial ecosystems evolve to maintain resilience, providing broadly applicable insights into fundamental microbial ecology.

In collaboration with The Yanomami Foundation and research partners, we analyzed a unique dataset of approximately 9 fecal samples collected from the Yanomami, an indigenous non-industrial population in the Amazon Rainforest of Venezuela and Brazil, providing rare representation of a minimally industrialized microbiome. Genomic DNA was sequenced using shotgun metagenomics. Publicly available fecal metagenomic datasets from non-industrial, transitional, and industrial populations were leveraged to bolster this analysis. Sequencing reads were put through a custom bioinformatics pipeline with a custom KRAKEN2 database. After filtering for ultra-low abundance reads, microbial taxa strongly associated with the non-industrial and industrial lifestyles. However, there 277 taxa were present in >95% of the samples across all populations and lifestyles. We have defined this global “core” as the ENDURE microbes (**E**xisting **N**aturally with **D**iverse and/or **U**nstable **R**esources and **E**nvironments), thereby, adding further context to the existing VANISH and BLoSSUM categories.

By leveraging rare access to Yanomami-derived samples, this project moves beyond descriptive surveys of the human gut microbiome by establishing a cross-cultural baseline of taxa that persist across drastic ecological gradients. ENDURE microbes provide a model to understand how certain taxa withstand ecological perturbations, colonize diverse hosts, and maintain functional stability. Ultimately, the outcomes of this project will advance fundamental microbiology by clarifying how microbe-microbe interactions and functional redundancy contribute to resilience of microbial ecosystems.

Section Type

AEM

S4 - Phage-phage and phage-antibiotic interactions dictate the efficacy of combination treatments targeting *Burkholderia glumae*



Brittany Supina[1], Dr. Jonathan Dennis[1]

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Bacterial pathogens infect all major food crops and represent a growing threat to global food production. As an alternative to antibiotics, which pose environmental risks and are often strictly regulated, naturally abundant bacterial viruses called bacteriophages (phages) have gained significant traction. Phage-driven lysis of pathogens requires successful interaction between phage receptor-binding-proteins and highly specific surface exposed structures (receptors) on their bacterial targets. The specificity of this interaction enables highly targeted pathogen killing, but mutations causing modification, masking, or loss of these receptors may prevent host recognition, conferring phage resistance. Receptor-mediated phage resistance poses a challenge to pathogen control and multi-phage treatments composed of phages targeting several different receptors are required to effectively suppress disease. Additionally, phage-antibiotic synergy offers a potential method to improve treatment outcomes and reduce antimicrobial resistance through diverse mechanisms such as enhanced phage production or antibiotic re-sensitization. However, potential for cross-resistance to multiple phages and antagonism within phage-antibiotic treatments necessitates investigation of phage-phage and phage-antibiotic combinations to inform the development of effective combinatorial treatments.

Burkholderia glumae is a high-risk bacterial pathogen that causes severe disease in rice, reducing grain yields and inflicting economic losses worldwide. Though several bacteriophages with promising *in planta* activity against *B. glumae* have been characterized, little is known about the receptor-mediated interactions between *B. glumae* phages and their bacterial hosts. Similarly, the potential for phage-phage or phage-antibiotic interactions remains unknown. In this study, we investigate the basis of cooperative phage-phage interactions between flagella-dependent jumbo bacteriophage, S13, and newly isolated *B. glumae* phages. Through whole genome sequencing and phenotypic analysis of phage-resistant mutants, we identify lipopolysaccharide (LPS) O-antigen as a common receptor amongst *B. glumae* phages and impaired flagellin glycosylation as a new phage-resistance pathway against flagella-dependent S13. However, phage combinations remain active against phage-resistant isolates arising through these distinct mutational pathways. Using phages S13 and Swan as a model, we investigate phage antibiotic interactions with oxolinic acid or kasugamycin, agriculturally applied antibiotics for which *B. glumae* resistance has been identified. Modified synogram assays show stoichiometry-dependent cooperation with oxolinic acid, and inhibition of phage activity for both individual and combination treatments by kasugamycin, suggesting that phage-antibiotic combinations may improve *B. glumae* disease control, but require situational consideration. Overall, these findings reveal the potential of combinatorial treatments for *B. glumae* control and shed light on the complex interactions between phages, antibiotics, and *B. glumae.*

Section Type

AEM

S5 - Comparative Analysis of *C. albicans* and *N. glabratus* in response to acute boric acid exposure



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Vaginal yeast infections affect 75% of people with vaginas at least once in their lives and are mostly caused by *Candida albicans* and *Nakaseomyces glabratus*. While most patients successfully clear the infection after treatment with fluconazole, a significant subset experience recurrent infections with no clear underlying cause. Boric acid is a commonly used second-line treatment for recurrent yeast infections. Previously, we performed an evolution experiment in which we sequentially passaged *C. albicans* in increasing concentrations of boric acid and isolated evolved populations that could grow in ~ 4× the ancestral inhibitory level of boric acid. Unexpectedly, this enhanced growth ability was not retained when evolved populations were revived from freezer stocks, suggesting that there is a constraint to evolving boric acid resistance in *C. albicans*. Gene expression changes associated with boric acid exposure have never been studied in fungi, and could help us understand the mechanism of action of boric acid and why there are constraints to evolving resistance to it. This study will look at changes in the gene expression associated with growth in high levels of boric acid in *C. albicans* and *N. glabratus*. As a first step, we performed RNA-seq in both species following acute exposure to species-specific inhibitory concentrations of boric acid. We found that 350 genes were upregulated and 233 were downregulated in *N. glabratus* and 557 genes were upregulated and 335 were downregulated in *C. albicans* in response to acute boric acid exposure. Out of those genes, 61 overlapped between the two species, suggesting some shared strategies between *N. glabratus* and *C. albicans*. GO enrichment analysis of these genes revealed strong enrichment for amino acid metabolic and biosynthetic processes, organic acid metabolism, purine biosynthesis, sulfur assimilation, and alcohol metabolic processes. Using this list of shared genes, our next step is to create mutants using CRISPR/Cas9 to understand their involvement in surviving in acute boric acid exposure. This represents the first study to examine gene expression changes associated with boric acid exposure in fungal pathogens and could help us understand the mechanism of action of boric acid.

Section Type

AEM

S6 - Microbial contributions influence dietary flexibility in a polyphagous agricultural insect pest



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The potato leafhopper, *Empoasca fabae*, is a highly destructive agricultural insect pest capable of feeding on over 200 plant species across North America, encompassing both woody and herbaceous hosts. This high degree of polyphagy contrasts sharply with the narrower host ranges of closely related Old World *Empoasca* species, indicating possible lineage-specific adaptations. Using a chromosome-level host genome and near complete metagenome-assembled genomes (MAGs) of host symbionts, we investigate bacterial contributions that influence host dietary expansion via horizontal gene transfer (HGT) and complementary carbohydrate metabolism.

A key finding is the horizontal acquisition of six glycoside hydrolase (GH) genes of predicted *Streptomyces* origin, where both phylogenetic analyses and gene architecture support bacterial-to-insect gene transfers. Three of these horizontally transferred genes, EfGH5-1, EfGH5-4, and Ef-GH5-5, encode modular domain GH proteins with dual catalytic sites, a configuration we suspect enhances polysaccharide processing efficiency. Transcriptomic profiling confirms that these modular domain GHs are highly expressed in the salivary glands and differentially regulated in response to host diet. Further heterologous expression and activity assays of recombinant EfGH5-1 validated degradation of cellulose- and galactomannan-based substrates, establishing that these enzymes likely serve a functional role in the expanded digestion of plant polysaccharides.

In parallel, we describe a restructuring of primary obligate symbionts. Two nutritional symbionts common of leafhoppers, *Candidatus* Karelsulcia and *Candidatus* Nasuia, are present and reported here for the first time within a member of the insect tribe Typhlocybinae. However, both show extensive degradation of essential amino acids (EAAs) pathways. To compensate for this loss of function, we propose replacement by a novel obligate symbiont found in this study, *Candidatus* Symbiopectobacterium sp. strain *Ef* (*Ca.* Sy*Ef*). *Ca.* Sy*Ef* was present across all insect samples tested and retains near-complete biosynthetic capacity for all ten EAAs. This symbiont replacement and metabolic complementation, where a single symbiont surpasses the combined nutritional contribution of ancestral relationships, likely contributes to *E. fabae*'s ability to feed from diverse host plants via nutrient buffering. Additionally, we describe four recurrent facultative Enterobacterales (*Enterobacter mori*, *Kosakonia cowanii*, *Pantoea agglomerans*, and *Pantoea ananatis*) that were consistently detected across four years of insect field collections with stable retention in an inbred colony. These facultative symbionts encode complementary carbohydrate-processing enzymes, including two putatively secreted GHs with demonstrated enzymatic activity.

Collectively, these findings support a multilayered nutritional architecture in *E. fabae* encompassing HGT, obligate symbiont replacement, and facultative metabolic supplementation. Through this work, we establish *E. fabae* as a model for understanding how bacterial contributions can drive insect dietary evolution and we highlight the broader role of microbiomes as active architects of ecological niche expansion in herbivorous insects.

Section Type: AEM

S7 - A viral strategy to disarm the Pseudomonas aeruginosa type IV pilus



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Bacteria-specific viruses, known as phages, have coexisted and evolved alongside their hosts for eons. These viruses hijack a host's cellular machinery to support their replication and dissemination throughout a population. To infect, phages must first bind an exposed primary receptor on the cell surface. From here, the phage can introduce its genetic material into the host for replication, but during this process, some phages will modify host cell surface architecture and/or physiology. For example, the *Pseudomonas aeruginosa*-specific phage DMS3 expresses Aqs1, which disables type IV pili (T4P) through an unknown mechanism. These structures are common primary phage receptors in this organism, and this mechanism helps to evade recognition by competing phages which also leverage pili to infect. T4P are not only phage receptors, however, but they also facilitate *P. aeruginosa* motility, tissue invasion, host colonization, and biofilm formation making them attractive targets for anti-virulence treatments. How then does Aqs1 disable T4P, and can we exploit phage-encoded systems to fight priority pathogens? To address this, we confirmed that Aqs1 binds to PilB, an essential homohexameric ATPase responsible for assembling and extending pilus filaments into extracellular space. Using protein modelling, we identified a putative Aqs1 binding region on PilB distal to the active site. Two Aqs1 side chains, known to be important for T4P inhibition, packed into a solvent-exposed hydrophobic patch on the PilB surface. We individually mutated some of the PilB side chains surrounding this patch which were potentially involved in coordinating Aqs1 to validate binding. Notably, PilB P228A and P229A substitutions had decreased Aqs1 interaction compared to wildtype. When we individually introduced these mutations onto the *P. aeruginosa* chromosome, Aqs1 expression no longer inhibited T4P-dependent twitching motility supporting the binding site in cells. Similarly, we saw a minimal reduction in the percentage of PilB P228A mutant cells producing fluorescently-labelled pili when Aqs1 was expressed, consistent with the retention of twitching in this background. To disable pilus function, Aqs1 expression delocalized PilB and a fluorescently-tagged regulatory protein from the cell poles, where T4P are made in this organism, thus preventing PilB from acting on T4P nanomachines. This suggested that Aqs1 was likely disrupting some critical PilB protein-protein interaction required for normal function. Consistent with this idea, adding Aqs1 to stable PilB hexamers induced the dissociation of these complexes into lower molecular weight species. Since PilB hexamerization is essential for function, with individual subunits lacking the necessary architecture for substrate and T4P machine recognition, we reasoned that loss of PilB polar retention was consistent with disruption of oligomerization. Our data emphasize specific recognition by Aqs1 of an essential PilB allosteric site. This provides a template to develop small molecules which mimic the Aqs1 mechanism of action, to act as PilB inhibitors to limit the virulence of priority pathogens like *P. aeruginosa*. By targeting the Aqs1-binding hydrophobic patch, such compounds may also avoid the toxicity associated with traditional active-site based ATPase inhibitors.

Section Type

MGCM

S8 - Control and activity of distinct isoforms of the nucleoid-associated protein LsrL in Streptomyces



Lauren Tiller[1], Vivian Ramirez[2], Xiafei Zhang[1], Lydia Freddolino[2], Marie Elliot[1]

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Streptomyces are soil-dwelling, Gram-positive bacteria known for their multicellular life cycle and their ability to produce a vast array of specialized metabolites, including many antibiotics. Specialized metabolites are produced by enzymes encoded by groups of colocalized genes on the chromosome, known as biosynthetic gene clusters, with each cluster directing the production of a specific metabolite. *Streptomyces* have the genetic capacity to produce far greater numbers of specialized metabolites than have been detected to date: in many cases, the products of these uncharacterized biosynthetic clusters are unknown, and in other instances, the clusters themselves are transcriptionally silent under laboratory conditions. How these clusters are controlled genetically is a major question in the field.

We have recently identified a major gatekeeper of specialized metabolism: the nucleoid-associated protein Lsr2. Lsr2 represses the expression of most specialized metabolic clusters in the model species *Streptomyces venezuelae*. Lsr2 is conserved across the Actinobacteria, while its paralog, LsrL, is found predominantly within the streptomycetes. Transcriptomic analyses of *lsr2, lsrL,* and double mutant strains (relative to wild type) suggested that LsrL may function to modulate the repressive activity of Lsr2. To test this possibility, we probed the DNA binding and interaction potential of these two proteins. Using bacterial two-hybrid analyses and immunoprecipitation experiments, we found that Lsr2 and LsrL can physically associate, supporting the potential for interesting functional interplay between these proteins. We explored their DNA binding used chromatin immunoprecipitation, followed by deep sequencing (ChIP-seq), to identify Lsr2 and LsrL DNA binding sites in *S. venezuelae* in both the presence and absence of the other protein. We found that Lsr2 and LsrL binding sites overlapped, and that Lsr2 binding increased in the absence of LsrL, supporting a modulatory role for LsrL. We confirmed the ability of Lsr2 and LsrL to bind these sites using *in vitro* electrophoretic mobility shift assays. Intriguingly, we found that *in vivo,* LsrL was unable to bind DNA in the absence of Lsr2 in *S. venezuelae*, which was inconsistent with our *in vitro* DNA-binding experiments. Western blotting revealed that LsrL existed as a larger protein isoform in the absence of Lsr2 (~23 kDa vs 15 kDa in wild type), and we hypothesize that this alternative form of LsrL is unable to bind DNA. Analysis of the 5' untranslated region of LsrL revealed the potential for an alternate, in-frame upstream start codon, and mutation of this putative start codon led to the loss of this larger LsrL product, suggesting that LsrL possesses at least two translation start sites, with translation of the larger variant only occurring in the absence of Lsr2. Our ChIP-seq data indicates that Lsr2 associates with the LsrL promoter region, and in its absence, it appears that this enables the transcription and translation of alternate forms of LsrL. These results suggest that *S. venezuelae* employs a unique mechanism to ensure coordination between nucleoid-associated proteins in the cell. These findings have the potential to allow us to better manipulate *Streptomyces* specialized metabolic cluster activity, and stimulate the production of novel bioactive compounds.

Section Type

MGCM

S9 - Beyond FarE: FarR functions as a global regulator of fatty acid stress adaptation, metabolism and virulence in *Staphylococcus aureus*



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Canonically, TetR family regulators are responsible for repressing their effectors. However, our lab has previously identified the TetR family regulator, FarR, which induces the resistance-nodulation-division efflux pump FarE for detoxification of unsaturated fatty acids in *Staphylococcus aureus*. Moreover, FarR variants have demonstrated repeated emergence in CC5, CC8 and CC97 lineages of methicillin-resistant *S. aureus* which confer enhanced linoleic acid resistance and increased *farE* expression. The selective adaptation of these variants may reveal a novel mechanism by which *S. aureus* alters fatty acid metabolism to survive under differing conditions, however, the global role of FarR in regulating these pathways remains poorly characterized. RNA sequencing was used to investigate the global regulatory role of FarR during mid-exponential growth with and without exposure to linoleic acid. In the absence of linoleic acid, USA300Δ*farR* exhibited 41 upregulated and 61 downregulated genes relative to USA300 (FDR < 0.01, $|\log_2 FC| \geq 1$). Reduced expression was observed for metabolic genes (*rocD, adhE gudB, SAUSA300_RS00930*), as well as stress-response genes associated with heme efflux (*hrtAB*) and heat shock (*grpE, clpB, hrcA, groL*). Under linoleic acid exposure, 22 genes were upregulated and 41 were downregulated in Δ*farR.* Genes downregulated in addition to *farE* included metabolic loci (*rocD, gudB*) and several secreted virulence factors (*coa, sbi, ecb, efb, hyl, nuc, lukGH, scb*). Regulatory interplay was suggested by reduced FarE production in mutants lacking *sigB, saeS* or *saeP,* supporting a broader role for FarE in coordinating fatty-acid stress responses with global stress and virulence networks. RNA-seq data also indicated reduced transcription of multiple secreted proteases (*sspA, sspB, sspC, aur*), which corresponded with phenotypic decreases in protease production that could be restored through complementation. Conversely, protease-deficient mutants exhibited markedly reduced FarE production, indicating a reciprocal relationship between protease activity and *farE* expression. Finally, FarR was investigated as a regulator of the fad operon that contributes to metabolism of exogenous fatty acids through β-oxidation. Although *farE* was not induced by palmitic acid, Δ*farR* showed increased susceptibility to both unsaturated and saturated fatty acids. Reporter assays and EMSA demonstrated that FarR directly binds the *fadX* promoter and modulates *fad* operon expression. Together, this work demonstrates a potential role for FarR as both an inducer and repressor to differentiate between unsaturated and saturated fatty acids in *S. aureus* for coordination of fatty-acid detoxification, metabolism, stress adaptation and virulence.

Section Type

I&I

S10 - Defining mechanisms of polyamine response in *K. pneumoniae* and *S. aureus* leads to discovery of novel inhibitors as potential antivirulence agents



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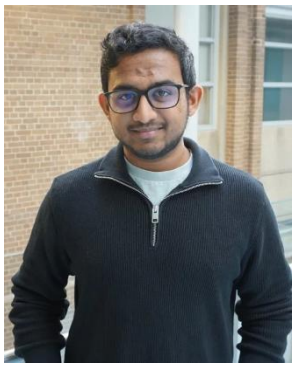
Drug-resistant *Klebsiella pneumoniae* and *Staphylococcus aureus* are listed by the World Health Organization as critical and high priority pathogens requiring an urgent need for the development of new treatment options. Chemicals, such as polyamines, present at the site of infection influence microbial virulence and response to antibiotics. In previous work, I performed a high-throughput drug screen and identified OES2-0017 and OES1-1087 which are broad-spectrum polyamine detoxification inhibitors. A chemogenomic screen suggested they target SpeG, a spermine/spermidine acetyltransferase that detoxifies polyamines. Through this work we also observed reduced *Salmonella* Typhimurium bacterial burden when treated with OES2-0017 or OES1-1087 in treated macrophages and a murine oral infection model, respectively, verifying that polyamine response inhibitors demonstrate antivirulence effects. The aim of this work is to identify the mechanism of action of both OES2-0017 and OES1-1087. Checkerboard assays between spermine and inhibitors showed synergy in *S. aureus* wild-type cells and a loss of synergy in a Δ*speG* mutant suggesting the target is SpeG. I purified SpeG for *in vitro* colorimetric enzymatic assays, which show inhibition of SpeG activity when treated with OES2-0017, but inhibition with OES1-1087 was weak with only a slight decrease in enzymatic activity observed. A more sensitive HPLC method confirmed SpeG inhibition by OES2-0017 but revealed no inhibition by OES1-1087. Therefore, I hypothesized that OES1-1087 was interacting with another polyamine response determinant that indirectly affects SpeG activity. To test this hypothesis I screened a sequence-defined transposon mutant library of *K. pneumoniae* containing 12288 mutants and identified 56 mutants, representing 32 genes, with increased susceptibility to spermine, including *speG* and *aceE,* which encodes the E1 component of pyruvate dehydrogenase. The pyruvate dehydrogenase complex produces acetyl-CoA, which is required for SpeG to acetylate and detoxify polyamines. Therefore, I hypothesized that a reduction in acetyl-CoA content may be impairing the ability of SpeG to detoxify polyamines. Both inhibitors synergize with spermine in the wild-type *K. pneumoniae,* and this synergy is lost in mutants of *speG* and *aceE.* The loss of synergy in both mutants suggests that either direct SpeG inhibition or reduced acetyl-CoA content are independently sufficient to sensitize the bacteria to spermine. I then quantified the cellular concentration of acetyl-CoA in wild-type *S. aureus* treated with sub-inhibitory concentrations of OES1-1087 and saw a reduction in acetyl-CoA concentration. Taken together, these results indicate that OES2-0017 is directly inhibiting SpeG, while OES1-1087 is indirectly inhibiting SpeG activity by reducing the cellular content of acetyl-CoA. This work is significant as it improves our understanding of cellular responses to host molecules in both *S. aureus* and *K. pneumoniae* and identifies novel polyamine response inhibitors that have the potential to reduce bacterial virulence or to be used on their own as novel antimicrobial agents.

Section Type

I&I

S11 - Defining the Molecular Details of Lysozyme Resistance



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Lysozyme is a key enzyme in the innate immune system that acts as the first line of defence against bacterial pathogens. This enzyme has also been exploited in various other industries, such as the agricultural, food, and pharmaceutical industries as an antimicrobial preservative. Most recently, lysozyme has been considered as a potential antibiotic replacement in animal feed in the agricultural industry, in attempts to mitigate the threat of antibiotic resistance correlated to the use of antibiotics in agriculture.

Lysozyme compromises the integrity of the bacterial cell wall and induces cell lysis through the hydrolytic cleavage of the primary cell wall component peptidoglycan (PG). Specifically, lysozyme hydrolyses the scissile β-1,4-glycosidic linkage between N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG); the two repeating monosaccharides that comprise PG. Despite its utility as an antibacterial agent, lysozyme's potency is limited against many bacteria because of PG modifications that resist degradation and overcome host innate immunity. The primary PG modifications that confer lysozyme resistance involve non-stoichiometric alterations of the glycan chain, including 2-*N*-deacetylation of NAG and C6-*O*-acetylation of NAM. The existing models for lysozyme resistance lack specific mechanistic detail, failing to explain the precise mode of action. For example, it is unknown whether resistance conferred by PG modification depends on impaired recognition at every subsite or only specific subsites within the active site cleft. Moreover, the current models also do not account for active-site variations among lysozyme orthologs. We hypothesize that these factors may play an important role in lysozyme resistance and have practical implications for the antimicrobial function of lysozyme.

In this work, we generated a panel of N-deacetylated and O-acetylated PG oligosaccharides to systematically investigate the precise molecular mechanism of lysozyme resistance. Using a liquid chromatography/mass spectrometry-based method (LC-MS), the hydrolytic activity of HEWL and HL was tested against a panel of PG octasaccharides $(NAM\text{-}NAG)_4$ containing non-stoichiometric amounts of O-acetyl or N-deacetyl substitutions to determine how lysozyme tolerates each modification at the six subsites of the active site. Upon determining which fragments were resistant to lysozyme, isothermal titration calorimetry (ITC) was used to determine whether inhibition results from reduced binding affinity or from non-productive binding interactions. Finally, the X-ray crystal structures of the complex with resistant PG fragments were analyzed to gain a structural basis for how *O*-acetyl and *N*-deacetyl PG interact with lysozyme's active site.

The molecular insights from this work have provided a clearer understanding of the mechanisms underlying lysozyme-PG interactions and support a revised model of protection by PG modifications. This has important implications for understanding how pathogens adapt to innate immunity and how resistance mechanisms mediate host-pathogen interactions. Our new insights into lysozyme's substrate specificity can inform its application for lysing diverse bacteria in research, biotechnology, food, and agriculture.

Section Type: I&I

S12 - Investigating the molecular mechanisms of Lodgepole Pine's resilience to the Mountain Pine Beetle and its associated fungal symbionts



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Mountain Pine Beetle (MPB; *Dendroctonus ponderosae*) is an invasive pest species affecting Lodgepole Pine (LP; *Pinus contorta*) in Canada. Along with its primary fungal associate, *Grosmannia clavigera,* a necrotrophic pathogen, MPB has devastated LP forests in Western Canada. While the MPB acts as the vector for this pathogen, *G. Clavigera* is a key component in driving host mortality by colonizing the phloem. Among the devastated tree stands, survivor trees offer the potential to study and understand resilient traits.

Using samples collected from F1 Progeny of MPB-killed and healthy LP trees, a custom SNP-probe & RNA-seq analyses found candidate gene targets for further characterization. To characterize the mechanisms involved in this host-fungus interaction via the candidate gene targets, corresponding *Arabidopsis thaliana* orthologs were identified and cloned, and homozygous T-DNA insertional knockout plant lines were acquired.

In parallel, efforts have been made to clone the putative MPB resilient gene targets from LP for complementation assays involving the model plant *Arabidopsis thaliana* and the necrotrophic pathogen *Botrytis cinerea*. Phenotypic screening using lesion quantification and histochemical staining was conducted on these different lines infected with *B. cinerea*.

With these targets involved in various plant cell processes, we undertook phosphoproteome and proteome analyses to better understand the potential molecular mechanisms underpinning MPB resilience to enable future breeding efforts. Our goal is to characterize the conserved pathways by doing so, this research will provide a molecular basis for tree breeding and future forest management strategies.

Section Type

I&I

STUDENT POSTER COMPETITION/ CONCOURS D'AFFICHES ÉTUDIANTES

Student competition abstracts will be separated into their own section of the poster board area (see numbering of abstracts in this section) and organized according to one of three CSM subsections: Applied & Environmental Microbiology (AEM), Infection & Immunity (I&I) and Molecular Genetics & Cellular Microbiology (MGCM). The poster number is located beside the title of the abstract and should be presented as follows:

- **SP1-6 numbered abstracts are presented on Wednesday June 17, 2026**
- **SP 7-12 numbered abstracts are presented on Thursday, June 18, 2026**

All posters will be presented in the **Education Gymnasium** (UAlberta campus, 87 Ave NW, Edmonton, AB T6G 2G5), on either the Wednesday or the Thursday Poster sessions (13:00-15:00 MST). Please refer to the CSM 2026 conference website for up-to-date scheduling changes:

https://event.fourwaves.com/csm2026/schedule

Poster presenters should stand by their posters for 1 hr according to the following schedule:

- **Wednesday June 17:** For the first hour SP1, SP3, and SP5 should stand by their poster and for second hour SP2, SP4, SP6 should stand by their poster.
- **Thursday, June 18:** For the first hour SP7, SP9, and SP11 should stand by their poster and for second hour SP8, SP10, SP12 should stand by their poster.

Posters should be placed by their respectively numbered poster board tag, according to their listed abstract ID number indicated in this booklet. Posters should be set up a few hours prior to their presentation and taken down after the poster session ends. Posters that have not been removed by the end of day, may be disposed of. The local organizing committee (LOC) and CSM are not responsible for any items kept at poster boards that may be lost or stolen during any sessions.

Les résumés des concours étudiants seront présentés dans une section distincte du panneau d'affichage (voir la numérotation des résumés dans cette section) et classés selon l'une des trois sous-sections de la SCM : Microbiologie appliquée et environnementale (AEM), Infection et immunité (I&I) et Génétique moléculaire et microbiologie cellulaire (MGCM). Le numéro de l'affiche est indiqué à côté du titre de l'affiche et doit être présenté comme suit :

- **Les résumés numérotés SP1 à SP6 seront présentés le mercredi 17 juin 2026.**
- **Les résumés numérotés SP7 à SP12 seront présentés le jeudi 18 juin 2026.**

Toutes les affiches seront présentées au gymnase **Education Gymnasium** (UAlberta campus, 87 Ave NW, Edmonton, AB T6G 2G5), le mercredi 17 juin ou le Jeudi 18 juin 2026 (de 13 h à

15 h HNE). Veuillez consulter le site web de la conférence SCM 2026 pour connaître le calendrier à jour (en cas de modification) :
https://event.fourwaves.com/csm2026/schedule

Les présentateurs de posters doivent rester debout pendant une heure, selon le calendrier suivant :

- **Mercredi 17 juin :** Pendant la première heure, les stagiaires SP1, SP3 et SP5 doivent se tenir près de leur affiche ; pendant la deuxième heure, ce sont les stagiaires SP2, SP4 et SP6 qui doivent se tenir près de la leur.
- **Jeudi 18 juin :** Pendant la première heure, les stagiaires SP7, SP9 et SP11 doivent se tenir près de leur affiche ; pendant la deuxième heure, ce sont les stagiaires SP8, SP10 et SP12 qui doivent se tenir près de la leur.

Les posters doivent être placés à côté de leur numéro d'étiquette sur le panneau d'affichage, conformément au numéro de résumé indiqué dans ce livret. Ils doivent être installés quelques heures avant leur présentation et démontés après la fin de la session. Les posters non retirés avant la fin de la journée seront jetés. Le comité d'organisation local (COL) et le CSM déclinent toute responsabilité en cas de perte ou de vol d'objets conservés sur les panneaux d'affichage.

SP1 - The genome of leafhopper-associated bacteria reveals the presence of potential biocontrol agents



Ariane Barrot[1, 2, 3, 4, 5, 6], Joshua Molligan[1, 2, 3, 4, 5, 6], Edel Pérez-López[1, 2, 3, 4, 5, 6]

[1]Université Laval, [2]Département de Phytologie, Faculté des sciences de l'agriculture et de l'alimentation, [3]Centre de recherche et d'innovation sur les végétaux (CRIV), [4]Institut de Biologie Intégrative et des Systèmes (IBIS), [5]L'Institut EDS, [6]Réseau québécois de recherche en agriculture durable (RQRAD)

In conventional agriculture, the use of synthetic pesticides is still the preferred strategy for pest management. However, these products face increasing scrutiny due to the growing prevalence of insecticide resistance and the negative impacts they have on the environment. In response, the search for alternative sustainable alternatives like the implementation of microbial control agents (MBCAs) has surged. Among these, the use of bacteria-based MBCAs is especially interesting due to their demonstrated efficacy and their established prevalence in the Canadian market space. In the province of Quebec, leafhoppers present an emerging threat to agriculture due to their migratory behavior and their capacity to vector phytopathogens. In response, this study aims to obtain the first bacterial collection based solely on bacteria found in leafhoppers to identify potential entomopathogen that could replace pesticides.

Here, we first captured leafhoppers from the fields of Quebec and identified them to their genus and if possible, without dissection, to their species. From there, the insects were grinded down in water to obtain starter plates over three different media, the MacConkey agar, the pseudomonas base agar, and the brain and heart infusion agar. These plates wielded a high diversity of morphologically different strains that were then individually reisolated. In a long-term conservation optic, these colonies were grown into liquid cultures to form glycerol stocks. Upon confirmation of preservation success, DNA was extracted to obtain amplicons of the 16S rRNA for Sanger sequencing. The sequences returned from sequencing were trimmed and blasted to obtain an identification. The clean sequences were also compared to each other using a local database to obtain a similarity matrix allowing us to choose samples from each cluster to send for long-read sequencing.

High molecular weight DNA was extracted from 12 isolates and sequenced using both Oxford Nanopore Technology and Illumina NovaSeq platforms (Plasmidsaurus, California, US). From there, we obtained a definitive identification of the strains and carried onto annotating the biosynthetic gene clusters (BGCs) present in our genomes. Among them, we noticed three interesting ones that could be useful for the biocontrol of leafhoppers that were present across multiple strains. The three predicted products are bacilisyn, an antibacterial that causes cell lysis and can be strengthened using genetic engineering. Lichenysin a cytotoxic and hemolytic protein that acts as a surfactant. Petrobactin, a siderophore, meaning it has a role of iron gathering within the host. It is also a requirement for a strain to be an animal pathogen.

This collection now expands our capacity for in vivo testing of identified isolates with MBCA capacity. Subsequent works will follow the testing of isolates, including assessing the relative expression of predicted BCG products. The expression of these products in our isolates and the confirmation of their entomopathogenic activity would allow for the development of modified strains to use in biocontrol. Upon success, this framework could be used for other pests to develop more specific control agents reducing the use of synthetic pesticides, and all the negative effects associated to their use.

Section Type: AEM

SP2 - UreC Genes Within Permafrost and Their Potential Applications for Soil Maintenance



Jarod McLeay[1], Camilla Nesbø[1], Scott Cocker[1], Brian Lanoil[1], Duane Froese[1]

[1]University of Alberta

Permafrost, soil frozen at or below 0 °C for at least two years, is a key component of the cryosphere and underlies much of the global North, storing a vast reservoir of organic carbon. Rising temperatures driven by global warming are accelerating permafrost thaw, increasing the risk of infrastructure instability. While engineering methods and monitoring are currently used to assess and mitigate these risks, more environmentally sustainable approaches are needed to maintain quality of life in northern communities. In temperate regions, microbially induced carbonate precipitation (MICP) has been used to stabilize soils, prevent liquefaction, sequester CO_2, and reduce the permeability of contaminants. This process typically involves either bioaugmentation, in which MICP-capable bacteria are cultured and introduced into soils, or biostimulation, which enhances conditions to promote the activity of native microbial populations. However, applying MICP in permafrost is challenging due to low temperatures and freeze-thaw cycles, which limit the survival of introduced bacteria. Biostimulation of native urease-producing microbes may therefore provide a more viable alternative, as these organisms are already adapted to local conditions. To evaluate this potential, we analyzed UreC gene diversity in metagenomic samples from both permafrost and temperate regions, including cores from the Yukon affected by freeze-thaw cycles associated with human development. Phylogenetic analysis revealed that while some UreC clades were specific to particular environments, most were widespread across both permafrost and temperate metagenomes. Extracted samples clustered with bacterial species previously identified as strong MICP producers. High UreC diversity was observed in permafrost metagenomes compared to non-permafrost metagenomes and extracted permafrost cores, although these differences were not statistically significant ($p = 0.248$). Bacterial genera were also largely shared across regions, with minimal variation by depth. However, differences in community composition were observed between disturbed and undisturbed permafrost samples. These findings suggest that native microbial communities in permafrost may support MICP through biostimulation. Further work is needed to compare MICP performance under temperate and permafrost conditions and to assess the impact of environmental disturbance. The widespread presence of urease genes also suggests a potential role for urea in nitrogen cycling within thawing permafrost environments.

Section Type

AEM

SP3 - A Genomic Blueprint for the Taxonomy and Metabolism of the Phylum “Zixibacterota”



Aman Verma[1], Ben Kilbrei[1], Marike Palmer[1]

[1]University of ManitobaUncultivated microorganisms revealed through metagenomics have substantially expanded the prokaryotic tree of life and uncovered previously unrecognized contributors to biogeochemical cycling. Among these, the phylum *“Zixibacterota”* (syn. “*Zixibacteria*”) represents a phylogenetically distinct bacterial lineage with a broad global distribution across subsurface environments spanning salinity and redox gradients. Heterotrophic lifestyles and respiratory versatility have been proposed for members of *“Zixibacterota”*, but these inferences are based on few genomic reconstructions. Consequently, evolutionary relationships, metabolic diversity, and ecological strategies within the phylum remain unresolved. Here, we establish a taxonomic framework for *“Zixibacterota”* and apply lineage-resolved metabolic reconstructions to assess whether key physiological traits are conserved at the phylum level or partitioned among lineages. Phylogenomic analyses based on conserved marker-gene sets and concatenated supermatrices resolved evolutionary relationships, with taxonomic boundaries supported by overall genome relatedness indices. Under this framework, *“Zixibacterota”* currently comprise one class, ten orders, twenty-one families, one hundred and eight genera, and two hundred and two species represented by at least one medium- or high-quality genome. Comparative analyses indicate that members of the phylum are likely diderm, rod-shaped cells with Type IV pilus systems. Central metabolism is uniformly heterotrophic and oxidative across the phylum, and energy conservation universally includes aerobic to microaerobic electron-transport chains, hydrogen metabolism, together with conserved iron acquisition and homeostasis. In contrast, alternative complex III-aa_3 oxidase systems, sulfate reduction, and polysaccharide degradation are likely restricted to specific lineages, which underpins ecological diversification across redox-stratified environments. Together, this study provides a genome-resolved framework for *“Zixibacterota”* evolution and ecology.

Section Type

AEM

SP4 - Genomic analysis of prophages from *Burkholderia gladioli* exposes extensive genetic diversity



Angelle Britton[1], Jonathan Dennis[1]

[1]University of AlbertaThe genetic plasticity of bacteria and bacteriophages (phages) has been influenced by the demands of co-evolution. In addition to rapid mutation to evade phage predation, many bacterial chromosomes harbor dormant prophages that can provide advantages to the host. Despite the metabolic cost of maintaining prophage DNA and the risk of future predation by host cell lysis, prophages are often retained because they encode genes that enhance host fitness. This process, known as “lysogenic conversion”, involves phage-encoded accessory genes that can provide beneficial functions to the host, including virulence factors, antibiotic resistance, toxin secretion, superinfection immunity or exclusion, anti-phage defense, and alterations to host metabolism. Prophages are widespread across bacterial genera, including all species of *Burkholderia*.

Burkholderia gladioli is a broad host range bacterium commonly found in water and soil, acting as a commensal or phytopathogen in plants such as onions, carnations, gladioli, and rice. *B. gladioli* can also act as an opportunistic pathogen in humans with cystic fibrosis or chronic granulomatous disease. Along with members of the closely related Burkholderia cepacia complex (Bcc), *B. gladioli* exhibits extensive antibiotic resistance, highlighting the need for alternative therapeutics. Phage therapy is a promising alternative to antibiotics because of the abundance of phages in the environment, host-specificity, and minimal side-effects. Although many phages infecting Bcc species have been characterized, relatively few phages specific to *B. gladioli* have been identified.

In this study, prophages were induced from *B. gladioli* strains using the DNA-damaging agent Mitomycin-C. Four phages (AB1, AB2, AB4, and AB6) were isolated and characterized. Morphological analysis by transmission electron microscopy (TEM) revealed that AB1, AB4, and AB6 exhibited myovirus morphology, while AB2 displayed siphovirus morphology. Host range analysis demonstrated that AB1, AB4, and AB6 had a narrow host range and were exclusively active against *B. gladioli* strains. The phage genomes were sequenced, and genomic characterization assessed genome organization, modular structure, protein coding sequences were categorized based on their putative function, and regions of interest were identified.

Gene cluster comparison of all known *B. gladioli* phage genomes demonstrated homology between distinct pairings, but lack of homology between these pairings showed their diversity. Proteome-based phylogenetic analysis, and gene-sharing networks supported this diversity and enabled the proposal of four novel viral genera. Additionally, each phage contained putative gene products consistent with lysogenic conversion, including genes potentially associated with virulence, anti-phage defense, and bacterial partitioning. These findings highlight the potential role of prophages in shaping bacterial fitness and phage susceptibility.

Overall, this work identified four novel *B. gladioli* prophages, nearly doubling the number of known phages for this species, and provides the first in-depth genomic characterization of these phages. By expanding our understanding of *B. gladioli* phage diversity and function, this research supports the development of phage-based therapeutics and provides a foundation for future studies investigating prophage-mediated effects on bacterial virulence, fitness, and phage interactions.

Section Type: AEM

SP5 - Soil Microbial Community Composition and Greenhouse Gas Flux Across the Forest-Tundra Ecotone in a Warming Arctic



Ke Ke Li[1], Christina Davis[1], Lyle G. Whyte[1]

[1]McGill University, Department of Natural Resource Sciences, QC, Canada

As the Arctic rapidly warms, natural treeline advancement is occurring in northern Canada, where boreal forests are gradually expanding into tundra areas. Although this shift may offer a unique opportunity to store carbon in the form of plant biomass, which can effectively absorb carbon dioxide (CO_2) from the atmosphere, it also presents environmental challenges and uncertainty. Arctic afforestation, which involves planting trees on land that has not recently been covered by forest, has been proposed as a strategy to utilize this land and help Canada achieve its net-zero emission goals by 2050. Despite growing interest in how Arctic ecosystems respond to global warming, few studies have specifically addressed the Arctic treeline ecotone. In particular, it remains unclear how soil microbes that contribute to nutrient cycling and greenhouse gas (GHG) emissions may respond to treeline advancement and potential afforestation.

This study investigates how forest-to-tundra ecotones and rising temperatures affect soil processes and GHG fluxes around Inuvik, Northwest Territories. Understanding whether treeline advancement or potential afforestation in tundra ecosystems will enhance carbon sequestration or unintentionally increase soil GHG emissions is critical. We specifically explored how ecosystem type (forest, treeline, and tundra) and warming influence soil microbial communities, CO_2 and CH_4 fluxes and soil nutrients using in situ gas flux measurements and a laboratory microcosm incubation experiment.

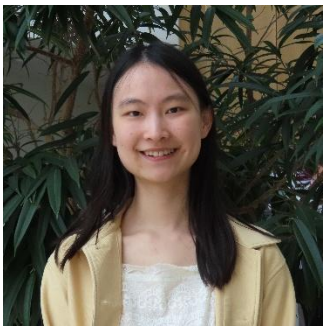
Preliminary results show clear ecosystem effects on soil CH_4 uptake and interannual variability in CO_2 flux. In 2024, tree soils showed the highest CO_2 flux relative to treeline and tundra soils, whereas in 2025 no clear site differences in CO_2 flux were detected. On the other hand, all three ecosystem types acted as a CH_4 sinks in both years, with tundra soils consistently showing the strongest CH_4 uptake. In the microcosm experiment, preliminary TC and TN plots suggested site and temperature dependent patterns, including a decline in TC in tree soils over 120 days and a possible stronger TN decrease under warming in treeline soils.

Together, these findings suggest that the loss of tundra soils due to the treeline expansion may compromise the regional soil's ability to act as a CH_4 sink and that forest expansion may not necessarily increase net soil GHG benefit. By addressing knowledge gaps in the potential impact of forest expansion and soil warming on microbial dynamics and GHG fluxes, this study will provide foundational insights into whether Arctic afforestation could be a feasible strategy for carbon sequestration, as well as its environmental impacts.

Section Type

AEM

SP6 - Disparate immunity proteins independently inactivate an antibacterial nuclease toxin



Vivian Liu[1, 2], Jake Colautti[1, 2], Youngchang Kim[3], John Whitney[1, 2, 4]

[1]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, L8S 4K1, Canada, [2]Michael DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Ontario, L8S 4K1, Canada, [3]Structural Biology Center, X-ray Science Division, Advanced Photon Source, Argonne National Laboratory, Lemont, Illinois, USA, [4]David Braley Center for Antibiotic Discovery, McMaster University, Hamilton, Ontario, L8S 4K1, Canada

Bacteria secrete a diverse arsenal of protein toxins that inhibit the growth of competing species. To avoid self-intoxication or targeting of genetically identical kin cells, antibacterial toxins are invariably encoded alongside cognate immunity proteins that typically bind to and occlude a toxin's active site, thus neutralizing its activity. Additional immunity proteins within a poly-immunity locus rarely target the same toxin, consistent with a predominantly one-to-one relationship between toxin-immunity pairs. Whether multiple immunity proteins can independently bind to and inhibit a single toxin, and how such systems are organized, remains poorly understood.

Here, we identify a T6SS-associated antibacterial toxin, which we term Tde5, in clinical isolates of *Pseudomonas aeruginosa*, and show that it is inhibited by two mechanistically distinct, adjacently encoded immunity proteins, Tdi5a and Tdi5b. Structural and bioinformatic analyses reveal that Tde5 is a member of the ββα-Me DNase superfamily, and we demonstrate that this enzyme inhibits bacterial growth through non-specific metal-dependent DNase activity mediated by conserved histidine residues. Notably, despite lacking sequence or predicted structural similarity to each other, expression of either Tdi5a or Tdi5b is sufficient to rescue toxin-mediated growth inhibition and inhibit Tde5-mediated DNase activity *in vitro*. Using biochemical and biophysical approaches, we show that Tdi5a and Tdi5b independently interact with Tde5 with 1:1 stoichiometry and low nanomolar affinities, and that a ternary complex forms between these three proteins, indicating that the two immunity factors bind to different surfaces of the toxin. To elucidate molecular contacts, we determined a 1.73 Å X-ray crystal structure of the Tde5-Tdi5a complex, which reveals that Tdi5a engages the toxin active site interface through predominantly electrostatic interactions. Together, these data suggest that Tdi5a and Tdi5b independently inactivate Tde5 by binding to catalytic and non-catalytic faces of the toxin, respectively. Finally, to assess the evolutionary conservation and broader prevalence of these proteins, bioinformatic analyses reveal that this dual-immunity system is a unique feature of *P. aeruginosa* and identify homologs of Tdi5b in non-toxic genomic contexts, suggesting that this protein fold may have been recently co-opted to protect against a nuclease toxin.

Together, these findings define a previously unrecognized dual-immunity mechanism and shed new light on the evolutionary flexibility of toxin-immunity systems. Such mechanism may enhance robustness against toxin escape mutations or broaden protection against related nucleases. Moreover, this dual protection architecture may enable the generation of novel protein-protein interaction specificities without affecting toxicity, potentially conferring a selective advantage for bacteria during competition with closely-related neighbours. Overall, this study suggests that some protective functions of poly-immunity loci may have been underappreciated, informing future studies of interbacterial competition and virulence.

Section Type

I&I

SP7 - Targeting a key regulator in bacterial pathogens: a key to precision antimicrobials



Julian Ortiz[1, 2, 3], M'Lynn Fisher[1, 2, 3], Natalie Lapa[1, 2, 3], Jonas Wong[1, 2, 3], Jimmy Lu[4], Jody Groenendyk[4], Joanne Lemieux[4], Marek Michalak[4], Wael Elhenawy[1, 2, 3, 5, 6]

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Antimicrobial resistance (AMR) is a critical and escalating global health concern that threatens our current repertoire of antibiotics. In consequence, there is a pressing need to develop antimicrobials with new modes of action. Importantly, these novel therapeutics should overcome the limitations associated with traditional antibiotics, including disruption of the microbiota and the emergence of resistance. This project addresses these challenges by developing pathoblockers (PB), a novel class of antimicrobials that target pathogens by inhibiting their virulence factors. By disarming rather than eliminating pathogens, PB exert less selective pressure for resistance and spare the health-associated microbiota. Our group and others have shown that pathogens belonging to Enterobacterales, such as *E. coli* and *Salmonella*, can use gut metabolites to outcompete commensals. Thus, we reasoned that targeting their Secondary Metabolism (SM), will curtail their fitness. In this project, we developed a high-throughput screening platform that identified two inhibitors (C1 and C2) of a key SM regulator in gut pathogens. Importantly, C1 and C2 inhibit the growth of several enteric pathogens under infection-relevant conditions without disturbing the microbiota. Using Surface Plasmon Resonance (SPR) and Electrophoretic Mobility Shift Assays (EMSA) we demonstrate that C1 and C2 bind specifically to the regulator and inhibit its function. Additionally, we have characterized the molecular interactions between C1 and the regulator. Finally, using a preclinical model of infection, we demonstrate the *in vivo* efficacy of compound C1. Together, our work highlights the potential of our screening pipeline to identify molecules that inhibit enteric pathogens while preserving the microbiota.

Section Type

I&I

SP8 - A PspC-Associated pH-Responsive Gene Cluster Linked to Cell Wall Homeostasis in Lachnospiraceae



Christian Pittao[1], Bradley Fitzgerald[1], Ethel Closa[1], Marissa Sim[1], Matthew Sorbara[1]

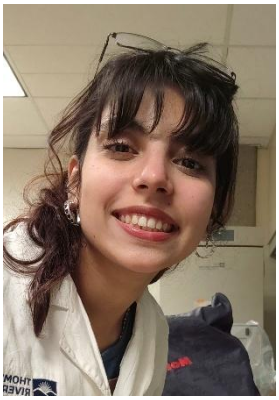
[1]University of Guelph

The human gastrointestinal tract harbours a dense, metabolically active microbial community that contributes to host physiology through nutrient processing, immune signalling, and maintenance of intestinal homeostasis. Within this environment, microorganisms are exposed to fluctuating physicochemical conditions, among which luminal pH is a key determinant of microbial growth and metabolic activity. Spatial and temporal variations in pH impose selective pressures on gut bacteria, necessitating adaptive mechanisms that preserve cellular integrity and ecological fitness. Although these stress responses are fundamental to microbial persistence, they remain insufficiently characterized in dominant commensal taxa. Members of the family *Lachnospiraceae*, which are abundant obligate anaerobes within the Bacillota phylum and important producers of short-chain fatty acids, have poorly defined molecular mechanisms for responding to environmental stressors such as pH variation. This study aimed to identify and functionally characterize pH-responsive genetic systems in representative *Lachnospiraceae* species. To investigate pH-responsive pathways in this lineage, transcriptional responses to pH were investigated in *Anaerostipes hadrus* using RNA sequencing. A contiguous four-gene locus was significantly upregulated at pH 7.00 compared to pH 6.25. This locus encodes a phage shock protein C homolog (pspC), two proteins of unknown function (annotated as Toastrack and HAAS), and a PadR family transcriptional regulator. Expression of this locus remained elevated at neutral pH in the presence of short-chain fatty acids, but metabolite supplementation alone did not alter transcription, indicating that regulation is driven by pH rather than metabolic by-products. Comparative genomic analysis demonstrated that the gene cluster is present across multiple *Lachnospiraceae* species, although the flanking regions vary. Sequence comparisons further revealed that pspC and PadR are relatively conserved, whereas the Toastrack and HAAS proteins exhibit greater divergence. Consistent with transcriptomic data, RT-qPCR across an expanded pH range confirmed that the cluster showed increasing expression toward alkaline pH in *A. hadrus,* whereas *Blautia luti* exhibited higher expression at acidic pH. Functional analysis of the cluster was performed by disrupting the *pspC* gene in *B. luti*. The mutant displayed reduced expression of the gene cluster and altered growth across pH conditions, with increased growth in acidic conditions and greater stability at neutral or alkaline pH. Additionally, mutant supernatants triggered less NOD2 reporter activation, suggesting altered peptidoglycan release, and metabolomic profiling revealed reduced levels of aspartate, lysine, and malate, which are linked to peptidoglycan biosynthesis. These results indicate shifts in metabolic flux and cell envelope dynamics. Collectively, these findings identify a putative pH-responsive signalling system conserved within *Lachnospiraceae* and provide a foundation for understanding how gut commensals adapt to fluctuating intestinal environments. Further investigation is necessary to elucidate how PspC-mediated environmental sensing influences cell envelope physiology and ecological fitness of *Lachnospiraceae* within the gut microbiota.

Section Type

MGCM

SP9 - Sample Preparation Methodology for Detection of Bacteriophage Structural Proteins using MALDI-TOF-MS



Maria Alejandra Rojas Rivero[1], Naowarat Cheeptham[1], Kingsley Donkor[1]

[1]Thompson Rivers University

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is widely used for rapid microbial identification due to its speed, cost-effectiveness, and minimal sample preparation compared to sequencing-based methods. However, its application in bacteriophage proteomics remains limited due to challenges in protein extraction, purification, and reproducibility. The objective of this study was to develop and evaluate a streamlined workflow for the preparation and identification of bacteriophage structural proteins using MALDI-TOF MS.

Two bacteriophage strains, EC1KELCTY and EC3KAMCTY, were propagated using *Escherichia coli* hosts through plate lysate preparation. Phage viability was confirmed using a small drop plaque assay, ensuring successful amplification prior to protein analysis. Phage lysates were denatured using SDS sample buffer containing dithiothreitol (DTT) and separated using 8% SDS-PAGE. Protein bands ranging from approximately 30 to 150 kDa were visualized using Coomassie Brilliant Blue staining and excised for in-gel trypsin digestion.

Following reduction and alkylation, peptides were extracted and analyzed by MALDI-TOF MS in the mass range of 1-4.5 kDa. Peptide mass fingerprints were generated and searched against the Swiss-Prot database using the MASCOT search engine. A bovine serum albumin (BSA) standard was included as a control to evaluate digestion efficiency and instrument performance.

Distinct protein banding patterns were observed for both bacteriophage strains, suggesting differences in structural protein composition. MALDI-TOF spectra showed detectable peptide signals across all samples, and database searches suggested the presence of phage-associated proteins, including integrase and terminase subunits. However, none of the identifications reached statistical significance, with MASCOT scores below the threshold (≥55). Additionally, the BSA control failed to produce correct identification, indicating limitations in sample preparation or peptide recovery.

These results suggest that while the developed workflow enables detection of bacteriophage-derived peptides, further optimization is required to improve identification confidence. Key limitations likely include low protein concentration, incomplete digestion, and interference during ionization. Future work will focus on improving protein concentration using spin column purification, enhancing peptide recovery through optimized extraction protocols, and incorporating desalting steps to improve spectral quality.

Overall, this study provides a foundational methodology for MALDI-TOF MS-based analysis of bacteriophage structural proteins and highlights critical factors influencing successful protein identification. Optimization of these parameters is essential for establishing MALDI-TOF MS as a reliable tool in bacteriophage proteomics and advancing its application in microbiological research.

Section Type

MGCM

SP10 - Identifying Genes Important for Salmonella Survival in Water



Rylan Bahrey[1, 2], Sylvia Li[2], Samantha Jelfimow[1, 2], Michelle Gerber[1, 2], Jessica Sparrow[2], Young Min Kwon[3], Mohamed Helmy[2], Aaron White[1, 2]

[1]Department of Biochemistry, Microbiology and Immunology, University of Saskatchewan, SK, CA, [2]Vaccine and Infectious Disease Organization, SK, CA, [3]Cell and Molecular Biology Program, University of Arkansas, AR, USA

Salmonella spp. are a major causative agent of human diarrheal disease worldwide, with up to a 30% mortality rate in developing countries. Despite this, researchers still do not understand the specifics of how Salmonella spp. are able to survive outside of a host. Survival in water and subsequent transmission are thought to be important for pathogenic success, but there is a gap in knowledge on how Salmonella can do this. Salmonella uses biofilms to survive desiccation, resist antimicrobials and increase survivability in general, therefore it has been hypothesized that Salmonella would use its biofilm capabilities to survive in water. To answer the question of what genes are important for Salmonella to survive in water, we built a transposon (Tn) library, where each strain contains a single Tn insertion, leading to inactivation of a gene. The entire collection of strains in the library represents every gene in the Salmonella genome. By inoculating the Salmonella Tn library into water and incubating for 50 days, we can determine what genes are most important for water survival. By comparing the Tn-library after 50 days in water (the output) to the starting Tn-library (the input) we are able to tell what genes are important. Through multiple experiments and replicates, we can differentiate between genes that are consistently identified versus those that appear randomly. I will describe the process of building and validating the Tn library and show data from our water survival experiments, including some of the first genes we have identified. Using a Tn library to investigate water survival will lead to a greater understanding of the mechanisms used by Salmonella to transmit and cause new infections.

Section Type

MGCM

SP11 - Exploring the role of altered phospholipid influence on the antimicrobial resistance phenotypes of *E. coli*



Ryan Darragh[1], Carmine Slipski[1], Patrick Chong[2], Peter McQueen[2], Garrett Westmacott[2], George Zhanel[1], Denice Bay[1]

[1]University of Manitoba, [2] National Microbiology Laboratory, Public Health Agency of Canada

INTRODUCTION: *Escherichia coli*, a Gram-negative facultative anaerobe, is a top-three antimicrobial-resistant (AMR) pathogen and a critical priority for the World Health Organization. Its outer membrane provides intrinsic resistance by requiring drugs to bypass two distinct lipid barriers. Despite nearly a century of drug development, the permeability and membrane entry mechanisms of many antimicrobials remain uncertain, though they appear associated with both protein- and lipid-based pathways. While resistance mechanisms like porin downregulation and efflux pump upregulation are well-documented, the specific role of membrane lipids requires further investigation.

Previous research suggests that lipids significantly impact resistance to both antibiotics and biocides (antiseptics/disinfectants). This project investigated how three phospholipid (PL) biosynthesis mutants—ΔpssA (lacking phosphatidylethanolamine; PE), Δ*clsABC* (lacking cardiolipin; CL), and Δ*pgsA* (70% reduction in phosphatidylglycerol; PG and CL)—affect *E. coli* efflux pump activity and intrinsic resistance. I **hypothesized** that PE, the dominant lipid in Gram-negative bacteria, is a primary driver of intrinsic AMR and is essential for the function of plasmid-inherited efflux pumps (MdfA, MdtK, EmrE, and AceI).

METHODS: We examined *E. coli* K-12 mutants AL95 (Δ*pssA*), UE54 (Δ*clsABC*), and BKT12 (Δ*pgsA*). Antimicrobial susceptibility testing (AST) was performed using standard 96-well broth microdilution against a comprehensive drug library from 12 major classes of Gram-negative selecting antibiotics (β-lactam, macrolide, tetracycline, chloramphenicol, quinolones, polymyxins) and Gram-positive selecting (glycopeptides/lipopeptides). Cloned efflux pumps (*mdfA, emrE, aceI, mdtK*) were expressed via a lactose-inducible P_{tac} pMS119EH vector and transformed into each mutant for AST. Protein expression was confirmed via Western blotting (His-tag detection). Additionally, Gram-stains of strains were analyzed via light microscopy to monitor phenotype changes.

RESULTS/ DISCUSSION: AST revealed that only the loss of PE (AL95) caused significant changes (>2-fold MIC) across diverse drug classes. AL95 showed a massive 2048-fold MIC increase for colistin and >8-fold increases for chloramphenicol, doxycycline, aztreonam, ciprofloxacin, and cefotaxime, suggesting that PE loss decreases susceptibility to many antibiotics. Conversely, AL95 became more susceptible to the Gram-positive-targeting drug vancomycin. No PL mutants showed altered MICs for biocides alone.

When expressing efflux pumps, the quaternary ammonium compound-selective pump EmrE showed the highest sensitivity to PL content. In AL95, EmrE expression led to increased susceptibility to ciprofloxacin and chlorhexidine, whereas in BKT12, it increased MICs 4-to-8-fold. The chlorhexidine-selective pump AceI showed a 2-to-4-fold reduction in resistance across all PL mutants, most notably in AL95. In contrast, MdfA and MdtK showed negligible MIC shifts.

Microscopy confirmed morphological impacts: AL95 cells were significantly elongated (>5 μm), while BKT12 cells were smaller (~1 μm) and exhibited clumping.

CONCLUSIONS: PE has an important role in *E. coli* intrinsic AMR, due to its significant impact on AMR profiles to a wide range of drug classes, supporting the hypothesis. PL mutants beyond just AL95 appear to make more profound impacts on biocide-selective efflux pump MIC values (*emrE, aceI*) as compared to antibiotic-selective efflux pumps tested (*mdfA, mdtK*). This finding may explain why variable MIC values are noted in Gram-negative species with different proportions of PE, PG, and CL. Lipid content may be an important factor to consider when inferring efflux-mediated resistance.

Section Type

MGCM

SP12 - Analysis of the role of phenotypic heterogeneity in Mycobacterium tuberculosis survival and persistence



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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is a major cause of global mortality by a single infectious agent, responsible for over one million deaths annually. Mtb is a facultative intracellular pathogen, evolved to persist in the host environment. Mtb infection results in a range of clinical outcomes, from latent infection to active disease. Even within an infected individual, Mtb exhibits remarkable heterogeneity due to the complex microenvironments it encounters, allowing it to adopt different physiological states. Studies have shown that Mtb uses phenotypic heterogeneity as bet-hedging strategy to survive immune and antibiotic stress.

Phenotypic heterogeneity refers to non-genetic variations within a clonal population, enabling microorganisms to thrive in fluctuating environments. This heterogeneity can be due to stochastic fluctuations in gene expression (“noise”), which can result from promoter transitions between active and inactive states, leading to bursts of transcription. These fluctuations cause significant variations in protein levels among individual cells, promoting phenotypic diversity. In addition, there are deterministic “sense-and-respond” signaling networks such as two-component systems (TCS), which detect external cues and activate downstream response regulators contributing towards this diversity.

We hypothesize that phenotypic plasticity in Mtb leads to phenotypic heterogeneity that, in turn, contributes to antibiotic persistence, latency-reactivation, and cell-fate decisions. To dissect both stochastic and deterministic contributors of this diversity, we aim to perform a global analysis of phenotypic noise to identify pathways driving heterogeneity and characterize the single-cell transcriptional dynamics of select TCS.

Towards this, we have constructed a genome-scale promoter library using a promoter-probe vector encoding two fluorescent proteins: destabilized mNeonGreen protein to report on promoter noise and stable mScarlet protein as an internal control downstream of a constitutive promoter. So far, we have assembled ~10000 strains and validated the library through fluorescence plate reader assays. We have captured transcriptional noise in the individual strains using flow cytometry and microscopy at single cell level.

In parallel, we have generated an MprA transcriptional reporter strain and validated its responsiveness using plate-reader measurements and quantitative RT-PCR, and we are currently analyzing its transcriptional dynamics using single cell time lapse microscopy under basal and physiologically relevant stress conditions.

The assembly, validation and, initial single-cell noise measurements of promoter library, along with construction and preliminary characterization of the MprA reporter will be presented.

Section Type

MGCM

FOME ABSTRACTS/ RÉSUMÉS SUR FOME

The 11th Annual Forum on Microbiology Undergraduate Education (FOME) posters will be available for viewing on Wednesday and Thursday, June 17-18, during the conference's two scheduled poster sessions. Please stop by poster numbers F1-F4 to learn more about what's new with the CSM Committee on Microbiology Undergraduate Education (CMUE) and discover how to participate in future events organized by this committee for next year's FOME.

Les posters du 11e Forum annuel sur l'enseignement de premier cycle en microbiologie (FOME) seront disponibles les mercredi 17 et jeudi 18 juin lors des deux sessions d'affiches prévues. Veuillez-vous arrêter au numéro d'affiche F1-F4 pour en savoir plus sur les nouveautés du Comité CSM sur l'enseignement de premier cycle en microbiologie (CMUE) et pour en savoir plus sur la façon de participer aux événements organisés par ce comité pour l'événement FOME de l'année prochaine.

F0 - Involving undergraduates in meaningful research: successes, tips, and pitfalls



Michael Charette[1]

[1]University of Brandon, Wpg, MB

Undergraduate research is a fundamental part of a university education. In the short term, it supplies graduate programs with intellectually curious and promising students who support the research and teaching missions of the university. In the long term, it creates a scientifically literate society and ensures the scientific workforce that is necessary for the health and economic development of Canada. However, providing undergraduates with a meaningful research experience can be tricky and is different from supervising graduate students. In this presentation, I will discuss some of my undergraduate research success stories and failures. Embedded throughout the talk will be my suggestions for providing undergraduates with a successful and meaningful research experience along with pitfalls to avoid.

Section Type

Invited speaker in FOME Workshop

F1 - Rapid and Low-Cost Sequencing of Phages from Single Plaques



Kieran Furlong[1], Celina Tanbari[1], Elizabeth Williams[1], Adam Rudner[1]

[1]University of Ottawa

Sequencing newly isolated bacteriophages remains a bottleneck in the SEA-PHAGES program and in most phage hunting workflows. In 2025, only 501 of the 3,007 phages discovered (13.8%) were sequenced using Illumina. This limitation stems from both cost ($100-$250 per sample), and the time and difficulty generating high-titer lysate and high-quality DNA. As a result, sequencing decisions are often driven by which phages amplify well and yield high-quality DNA, rather than by scientific interest, leaving some phage clusters underrepresented or undiscovered.

To address these limitations, we implemented an accessible, low-cost Nanopore workflow in our SEA-PHAGES 2025 discovery course. Using a rapid PCR barcoding protocol (Fox et al.), we sequence directly from single plaques and multiplex up to 96 phages in a one-day run. In our discovery course, we obtained complete or partial genomes for 62 of 63 phages and used these data to guide selection for Illumina sequencing.

This protocol should, in principle, fail to sequence the genomic termini, as it requires two independent transposition events in a linear genome. Nonetheless, we have obtained complete genomes from many phages with cohesive ends, though coverage is often reduced across the terminal junction. Our preliminary results suggest that ligation to create circular genomes may increase coverage across genomic ends, improving assembly of complete termini with minimal additional work.

Because this method sequences phage genomes from plaques we have also inverted the standard phage hunting workflow to increase the diversity of our phage collection. In a summer phage hunt targeting *Arthrobacter* hosts, we sequenced 118 plaques and archived 9 unique phages, while in another hunt targeting *Staphylococcal* hosts, we sequenced 95 plaques and archived 6 unique phages.

Although ligation and transposase-based Nanopore workflows can sequence raw phage DNA, PCR barcoding offers advantages: it is less dependent on template quality, generates more DNA for sequencing, and removes DNA modifications that interfere with nanopore detection. For example, mycophage Payton, whose genome is heavily modified, is refractory to both Illumina and direct Nanopore sequencing. We have shown its modifications inhibit PCR, but with sufficient amplification cycles we obtained a complete genome and identified Payton as a unique cluster AI phage.

To support broader adoption, we are developing an Observable platform for rapid base-calling, read quality assessment, genome assembly, and evaluation of assembly completeness. We are also developing DIY reagents that reduce sequencing costs to ~$5 per phage. The workflow's minimal sample prep makes it suitable for undergraduate research and rapid sequencing of phage mutants without high-titer lysates.

Reference

Fox, B., Chahal, J. & Lypaczewski, P. DNA extraction free whole genome sequencing of bacteriophage genomes from a single plaque. BioRxiv (2025) https://doi.org/10.1101/2025.10.21.683754

Section Type

Abstract selected for FOME Workshop Presentation & Poster

F2 - Artificial Intelligence-enhanced engaged learning in microbiology



Kinga Kowalewska-Grochowska[1, 2]

[1]University of Alberta, [2]Alberta Precision Laboratories

Engaged learning is an interactive approach where students are invested in the educational process through cognitive, emotional, and behavioral involvement instead of passive listening, in order to foster curiosity, motivation, and practical application. This approach leads to better academic performance, and lower dropout rates.

Artificial Intelligence (AI) has increasingly been playing an important role in education in current economic climate at schools and universities; however, lack of human interaction in primarily technology-driven settings can hamper students' social and emotional development. Thus, a blended approach balancing technology with traditional, human-centred teaching provides optimal teaching strategy.

We have designed a series of microbiology teaching sessions based on simulations or scenario-based learning principles to bridge the gap between theoretical knowledge and practical application. Students engage with problems in a specific context - rather than abstract concepts; this allows them to acquire the knowledge that is more readily transferable to genuine professional situation

Each session starts with a key story or scenario based in reality with multiple clues hidden in the body of the story. The session is then paused to present the learners with a set of questions they need to answer before moving on to the next component. During the pause, they are offered a possibility to work through questions in a group setting (or individually) as well as consult available resources (including their cell phones). Depending on the scenario, the scenario challenges may include “what additional questions would you like to ask at this moment to help guide you through your journey?” with the instructor providing necessary answers to help student along (but without jeopardizing the learning process)

Upon completion of this component, the scenario/story continues, revealing the successive sequence of events, or new information stemming from the questions asked earlier, and the process repeats in a similar question-answer-further developments manner, until case/simulation scenario is completed and final conclusions revealed.

The process also involves diverting into other (sometimes unexpected) aspects of the main story challenge (eg discussion of patient case of antrax re-directed to consider issues of laboratory safety, or environmental contamination and bioterrorism) - thus utilizing branching paths to teach complex, nuanced topics.

To maintain student engagement, the scenarios involve use of AI human avatars in short MP4 video clips either role playing or providing verbal or visual information; there, the words spoken as well as body language and external appearance may provide clues necessary to use in problem-solving. These videos are incorporated in the teaching story along with still images that deliver specific AI generated voice messages, or short concept - centered educational videos. This allows for multimodal learning aiming to address students’ diverse learning styles.

Last but not least, use of AI avatars featuring figures of different ethnicities or speaking with different accents ensures diversity and inclusion in undergraduate education, often under represented or ignored in today’s education.

Section Type

Abstract selected for FOME Workshop Presentation

F3 - The flip side of the pandemic: a road map to build a flipped classroom for upper year microbiology courses

Yannick DN Tremblay[1], Jenny-Lee Thomassin[1]

[1]Department of Biochemistry, Microbiology and Immunology, University of Saskatchewan

The COVID-19 pandemic forced university instructors to rapidly develop online courses that integrated aspects of traditional in-person teaching with recorded videos. The transition to in-person teaching required flexibility and the ability to rapidly alternate between in person classroom teaching and online learning. This period of uncertainty created opportunities for instructors to explore and develop curricula that extended beyond traditional modes of delivery. Here we share the lessons learned and examine the impact of various pedagogical interventions used in the development of an upper-year, flipped microbiology course guided by the principles of universal design.

Section Type

Abstract selected for FOME Workshop Presentation & Poster

F4 - Pandemic-proofing clinical microbiology education: A flipped curriculum and clinical reasoning framework for medical lab trainees

Natalie Marshall[1, 2], Jelena Holovati[1, 3]

[1]Division of Diagnostic & Applied Microbiology, Department of Laboratory Medicine & Pathology, University of Alberta, Edmonton, AB, [2]Public Health Laboratory (ProvLab), Alberta Precision Labs, Edmonton, AB, [3]Stem Cells, Canadian Blood Services, Ottawa, ON

The Problem: The COVID-19 pandemic exposed a key challenge in microbiology education: Public health laboratory professionals were needed both to meet surging testing demands and to train the future workforce. This strain was compounded by a shortage of medical laboratory technologists (MLTs). Together, these pressures highlighted the need for educational models that can continue during major disruptions without sustained, high-demand access to expert instructors.

Solution 1: Course Design: To address this, we developed a “pandemic-proof” approach to teaching public health microbiology for MLT trainees. The first component was the course design itself: a virtual, flipped-classroom format designed for flexible delivery. Core content was provided through curated asynchronous materials, while synchronous virtual sessions focused on discussion and applied learning. This design aimed to preserve learner access to specialized expertise while increasing flexibility, scalability, and resilience during periods of high service demand or limited in-person teaching capacity.

Solution 2: Clinical Reasoning Framework: The second component was an educational framework to support clinical reasoning, serving as the intellectual scaffold for higher-order learning. Rather than emphasizing memorization of organisms or tests, the framework helped learners connect clinical syndromes and disease processes to specimen selection and test choice. Team-based exercises and case discussions were used to support structured application of these concepts.

Why This Matters: Together, these elements offer a practical way to sustain high-quality microbiology education during periods of disruption while also promoting deeper learning in diagnostic reasoning and test utilization. This approach may be useful for microbiology educators seeking more flexible teaching models and clearer methods to teach applied clinical reasoning in clinical microbiology.

Section Type

FOME Poster

EQUITY DIVERSITY AND INCLUSION ABSTRACTS/ RÉSUMÉS SUR L'ÉQUITÉ, LA DIVERSITÉ ET L'INCLUSION

The Canadian Society of Microbiologists (CSM) recognizes and promotes Equity, Diversity, and Inclusion (EDI) in all of its activities including but not limited to membership, leadership, annual conference representation, and mentoring. We acknowledge the historical and ongoing intersectional discrimination of marginalized peoples in Canadian society, and within science in particular. In all our activities, we strive to promote and maintain a sense of welcoming and belonging for Indigenous Peoples, women, persons with disabilities, racialized people, 2SLGBTQ+ members, and other under-represented and marginalized groups. As part of our goals, this section provides a listing of the first inaugural showcase of abstracts submitted to highlight and promote EDI by the CSM membership at this year's conference in both poster sessions held June 17-18.

La Société canadienne des microbiologistes (SCM) reconnaît et promeut l'équité, la diversité et l'inclusion (EDI) dans toutes ses activités, y compris, mais sans s'y limiter, l'adhésion, le leadership, la représentation au congrès annuel et le mentorat. Nous reconnaissons la discrimination intersectionnelle historique et persistante des personnes marginalisées dans la société canadienne, et dans le milieu scientifique en particulier. Dans toutes nos activités, nous nous efforçons de promouvoir et de maintenir un sentiment d'accueil et d'appartenance pour les peuples autochtones, les femmes, les personnes handicapées, les personnes racialisées, les membres 2SLGBTQ+ et les autres groupes sous-représentés et marginalisés. Dans le cadre de nos objectifs, cette section présente la première présentation inaugurale des résumés soumis par les membres de la SCM pour souligner et promouvoir l'EDI lors des deux séances d'affiches du congrès de cette année, qui se sont tenues les 17 et 18 juin.

E0 - Science and Public Health Advocacy in Uncertain Times

Amalia Corby[1]

[1]American Society for Microbiology

This interactive session will provide an opportunity for conference participants to hear from the American Society for Microbiology on the state of US science policy and funding, advocacy basics, and engagement opportunities for the microbiology community. Learn about recent advocacy challenges and discuss what we can do to partner across borders and globally, to stay involved in discussions for sustainable change.

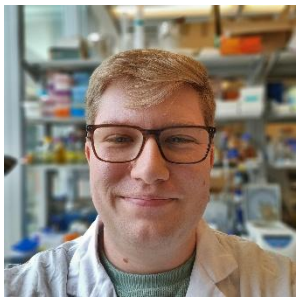
Section Type

EDIA Invited Speaker

E1 - Pride in Microbiology: Building a Global Community for 2SLGBTQIA+ Microbiologists

Landon Getz[1], Edel Pérez-López[2], Bruno de Oliveira[3], Katie Barnes[4], Nicholas di Cologna[5]

[1]Department of Biochemistry, Temerty Faculty of Medicine, University of Toronto, [2]Department of Phytology, Université Laval, Quebec, Canada, [3]Department of Microbiology and Parasitology, Fluminense Federal University, Niterói, Brazil, [4]Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, USA, [5]Biological Sciences Department, State University of New York at Albany, Albany, NY, USA



2SLGBTQIA+ microbiologists have largely navigated their careers in isolation. Recent STEM-specific data make the cost of that isolation clear: LGBTQ+ people are 17-21% less represented in STEM than statistically expected, almost 50% more likely to consider leaving the profession, and LGBTQ+ undergraduates in STEM are retained at lower rates than their peers despite participating in undergraduate research at higher rates. While many universities have embraced equity, diversity, and inclusion in principle, in practice, the connection, peer support, and professional development opportunities for 2SLGBTQIA+ microbiologists remain limited.

Pride in Microbiology (PiM) was founded in 2023 to build that infrastructure. We are an international, researcher-led organisation with three aims: build visible community across geographic and career boundaries, deliver professional development that accounts for the specific barriers 2SLGBTQIA+ microbiologists face, and work with established societies to embed inclusive practice into the discipline itself.

Nearly three years in, PiM has over 350 members across at least 20 countries and has initiated collaborative work with five major microbiological societies. Together, this has enabled us to host symposia and networking events, build peer support networks and a “wall-of-faces” visibility campaign, and facilitate dedicated EDI sessions at annual meetings. Our core activities include virtual symposia featuring 2SLGBTQIA+ microbiologists discussing their science and their careers, a mentorship network, called Pride in Microbiology Connections, providing targeted academic peer support, and regular network connection events such as Coffee Hours and Game Nights. This is all facilitated through an online network using email lists and Discord.

Feedback from members has been consistent on one point in particular: PiM is a space where people do not have to separate their scientific identity from the rest of who they are. Trainees report both personal validation and increased confidence in pursuing academic careers after engaging with visible queer faculty through our programming. In 2026, Pride in Microbiology was honored with the Microbiology Society’s Equity, Diversity, and Inclusion Prize, further validating the value of this work.

Broadly, community-led, researcher-driven organisations do something institutions often struggle to achieve: they build trust, convene honest conversation, and sustain the long-term community that keeps minoritised scientists in the discipline. They are a complement to institutional EDI work, not a substitute for it, and the two function best in partnership. We invite CSM attendees to engage with PiM and explore how we can work together to build a better microbiology discipline for everyone.

Section Type

EDIA & Speaker in Symposium 6 – Microbiology & Society June 17, 2026

GENERAL ABSTRACT POSTER GUIDELINES/ DIRECTIVES GÉNÉRALES POUR LES AFFICHES RÉSUMÉ

All abstracts listed in the following three CSM subsections: Applied & Environmental Microbiology (AEM), Infection & Immunity (I&I) and Molecular Genetics & Cellular Microbiology (MGCM) will be scheduled for a poster presentation as described below. The poster number is the same number used for the abstract number and it is located beside the title of the main abstract. Posters should be presented on the following dates:

- **Odd-numbered posters are presented on Wednesday, June 17, 2026**
- **Even-numbered posters are presented on Thursday, June 18, 2026**

All posters will be presented in **Education Gymnasium** (UAlberta campus, 87 Ave NW, Edmonton, AB T6G 2G5), on either Wednesday, June 17, and Thursday, June 18, 2026 (13:00-15:00 MST). Please refer to the CSM 2026 UAlberta conference website for up-to-date scheduling (if changes occur): https://event.fourwaves.com/csm2026/schedule.

Poster presenters should stand by their posters for 1 hr according to the following schedule:

- **Wednesday June 17:** The first hour is for presenters to stand by poster numbers ending in 1, 5, 9 and the second hour is for poster presenters with numbers ending in 3 or 7.
- **Thursday, June 18:** The first hour is for presenters to stand by poster numbers ending in 0, 4, 8 and the second hour is for poster presenters with numbers ending in 2 or 6.

Posters should be placed by their respectively numbered poster board tag number according to their listed abstract number in this booklet. Posters should be set up a few hours prior to their presentation and taken down after the poster session ends. Posters that have not been removed by the end of day will be disposed of. The LOC and CSM are not responsible for any items kept at poster boards that may be lost or stolen.

Abstracts presented below are listed according to their abstract submission identification number according to each section. We encourage readers to look for abstracts using the “FIND” search function and also perform a keyword search to find abstracts. Underlined authors represent the presenting abstract/ poster author.

Tous les résumés des trois sous-sections suivantes de la SCM : Microbiologie appliquée et environnementale (AEM), Infection et immunité (I&I) et Génétique moléculaire et microbiologie cellulaire (MGCM) feront l’objet d’une présentation par affiche, comme indiqué ci-dessous. Le numéro de l’affiche est le même que celui du résumé et se trouve à côté du titre du résumé principal. Les affiches doivent être présentées aux dates suivantes :

- **Les affiches impaires sont présentées le mercredi 17 juin 2026**
- **Les affiches paires sont présentées le jeudi 18 juin 2026**

Toutes les affiches seront présentées au **gymnase Education** (UAlberta campus, 87 Ave NW, Edmonton, AB T6G 2G5), le mercredi 17 juin ou le jeudi 18 juin 2026 (de 13 h à 15 h HNE). Veuillez consulter le site web de la conférence SCM 2026 de UAlberta pour connaître le calendrier à jour (en cas de modification) :

https://event.fourwaves.com/csm2026/schedule.

Les présentateurs de posters doivent rester debout pendant une heure, selon le calendrier suivant :

- **Mercredi 17 juin** : La première heure est réservée aux présentateurs qui se terminent par 1, 5 et 9, et la deuxième heure aux présentateurs qui se terminent par 3 et 7.
- **Jeudi 18 juin** : La première heure est réservée aux présentateurs qui se terminent par 0, 4 et 8, et la deuxième heure aux présentateurs qui se terminent par 2 et 6.

Les posters doivent être placés à côté de leur numéro d'étiquette sur le panneau d'affichage, conformément au numéro de résumé indiqué dans ce livret. Les posters doivent être installés quelques heures avant leur présentation et démontés après la fin de la session. Les posters non retirés avant la fin de la journée seront jetés. Le SCM déclinent toute responsabilité en cas de perte ou de vol d'objets conservés sur les panneaux d'affichage.

Les résumés présentés ci-dessous sont classés par numéro d'identification de soumission pour chaque section. Nous encourageons les lecteurs à rechercher des résumés à l'aide de la fonction « TROUVER » et à effectuer une recherche par mot-clé. Les auteurs soulignés représentent l'auteur du résumé/affiche.

INFECTION & IMMUNITY (I&I) ABSTRACTS/ RÉSUMÉS SUR L'INFECTION ET L'IMMUNITÉ (I&I)

25 - Advancing Rapid, Culture-Free screening for Uropathogens via Lipidomics

Linda Nartey[1, 2], Mert Pekcan[3], Victor Yuen[4, 5], Pamela Kibsey[4, 5], Robert Ernst[6], Michael Chen[4, 5], David Goodlett[1, 2]

[1]Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, V8P5C2, Canada, [2]Genome British Columbia Proteomics Centre, University of Victoria, Victoria, BC, V8Z7Z8, Canada, [3]Faculty of Veterinary Medicine, Department of Biochemistry. Ankara University, Ankara, Turkey, [4]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, V6T1Z4, Canada, [5]Department of Laboratory Medicine and Pathology, Vancouver Island Health Authority, BC, V8R 1J8, Canada, [6]Department of Microbial Pathogenesis, University of Maryland, Baltimore, 21201, MD. USA

Urinary tract infections (UTIs) are a major global health concern, and rapid, accurate pathogen detection is critical for timely treatment. Current clinical diagnosis relies on culture dependent workflows that are slow (18-24 hours) and labor-intensive. Protein-based MALDI TOF MS, although powerful, also depends on culture and are therefore similarly time consuming. Thus, no validated, culture-independent method currently exists to identify uropathogens directly from clinical samples. A faster workflow for pathogen identification that is direct from specimens, maintains the same accuracy as existing workflows, improve patient outcomes, and reduce healthcare costs is essential. We applied the Fast Lipid Analysis Technique (FLAT) to 402 outpatient urine samples. FLAT performs direct lipid extraction on a MALDI plate, generating microbial lipid “barcodes” for pathogen identification. Briefly, 1 µL of urine was spotted on a MALDI plate, acidified, incubated at 110 °C for 30 min, rinsed, and treated with norharmane matrix prior to analysis in negative linear ion mode on a Bruker Microflex. The diagnostic performance of FLAT was compared to standard urine culture (protein biotyper identification) and urinalysis. To improve Gram-positive detection, we incorporated lysozyme pretreatment to disrupt the peptidoglycan layer prior to lipid extraction to substantially improve Gram-positive detection. FLAT correctly identified common uropathogens and demonstrated 99% agreement with urine culture for negative samples, outperforming urinalysis (37% agreement). Negative UTIs were rapidly ruled out in 77% of cases, reducing culture reliance. While Gram-negative detection was strong, early FLAT workflows underperformed for Gram-positive organisms due to limited cardiolipin release. Lysozyme pre-treatment increased Gram-positive detection to 95% in a cohort of 76 culture-confirmed samples, without compromising Gram-negative detection, enabling reliable identification of both lipid classes in a single workflow. FLAT enables rapid, accurate, culture-independent microbial identification directly from patient urine, reducing diagnostic turnaround time by ~90%. FLAT was further extended to non urine specimens, including throat and vaginal swabs, demonstrating strong performance in detecting Streptococcus sp with sensitivity and specificity of 90% and 85%, respectively, supporting its broader applicability across clinical sample types. This integrated lipidomic workflow provides a robust framework for next-generation UTI diagnostics, supporting timely clinical decision-making and improved public health outcomes.

Section Type

Poster - I&I

30 - Discovery of an antivirulent therapy targeting streptococcal pyrogenic exotoxin A (SpeA) in *Streptococcus pyogenes*

Myia Blanchet[1], Amanda Marple[1], John McCormick[1]

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Streptococcus pyogenes is a Gram-positive human-specific pathogenic bacterium that is known to colonize the upper respiratory tract and the skin. Colonization with *S. pyogenes* within these regions can cause a range of both superficial and invasive clinical manifestations. *S. pyogenes* encodes a family of exotoxins known as superantigens (SAgs) that cause widespread activation of naïve T cells. This activation can stimulate an excessive release of proinflammatory cytokines, creating an environment that is conducive to colonization. Streptococcal pyrogenic exotoxin A (SpeA) is one of 16 SAgs that are encoded by *S. pyogenes*. Its expression has been linked to the development of both superficial and invasive infections including pharyngitis, scarlet fever and STSS which characteristically has a high mortality rate (23-44%). In addition, previous work in our lab has shown that SpeA is required for colonization of the nasopharynx. As antibiotic resistance is on the rise, SpeA's impact on *S. pyogenes* disease manifestation and colonization makes it a promising target for future antivirulent therapies as an alternative to the current antibiotic treatments. Therefore, **we hypothesize that disruption of *speA* regulation via antivirulent intervention will decrease *S. pyogenes* colonization and disease manifestation.** To elucidate this, we first sought to identify potential regulators of *speA* that could be used as future targets of antivirulent therapy. A previously developed *S. pyogenes* MGAS8232 transposon mutant library was screened using a newly developed capture ELISA against SpeA. Of the 576 screened mutants, five mutants showed a decrease in SpeA expression compared to wildtype and two underwent whole genome sequencing to determine which genes were disrupted. Whole genome sequencing of both mutants revealed only a single transposon insertion in each genome disrupting gene regions which both encode uncharacterized hypothetical proteins. A second approach to identify regulators of *speA* involved the use of a DNA-protein pull down assay against the *speA* promoter. DNA-binding proteins were evaluated by SDS-PAGE and identified by liquid chromatography mass spectrometry (LC-MS). Mass spectrometry identified a total of 352 DNA-binding proteins between both DNA probes (*speA* and control), with 159 proteins being found more abundantly from the *speA* promoter. Eight of the 159 proteins were identified as transcriptional regulators, with several other identified proteins being potential regulator candidates. Identified regulators will be assessed for their ability to directly regulate *speA* using an Electrophoretic Mobility Shift Assays (EMSA) with the *speA* promoter as the DNA probe. In addition, deletions will be generated in selected regulators. The impact on *speA* expression following loss of each regulator will be assessed through luminescence assays, anti-SpeA ELISA, and anti-SpeA Western blotting. Overall, this work will provide new fundamental insights into the regulatory pathways responsible for *speA* expression. This in turn can help in the development of an antivirulent therapy that targets SpeA production in patients afflicted with both superficial and invasive *S. pyogenes* infections, thereby decreasing the global burden of this prominent pathogen.

Gen abstract type

Poster

Section Type

I&I

39 - Clinically-relevant altered antibiotic responses and mechanisms of β-lactam sensitization of MRSA in cystic fibrosis artificial sputum

Tyler Hamelin[1], Ali Molaeitabari[1], Marc MacKinnon[1], Tanya Dahms[1], Omar El-Halfawy[1]

[1]University of Regina

Cystic fibrosis (CF) is the most common autosomal recessive disorder amongst the Caucasian population, causing a buildup of thick mucus that leaves patients vulnerable to bacterial infections. Up to 80% of pediatric CF patients are colonized by the Gram-positive pathogen *Staphylococcus aureus*, which is associated with poor prognosis, deteriorated lung functions, and other negative clinical outcomes. Current *in vitro* antimicrobial susceptibility tests (AST) rely on standardized culture media, namely Mueller Hinton broth (MHB), which does not adequately reflect the microenvironment present in CF patient's sputum. Standard AST results often do not correlate with antibiotic therapeutic outcomes, especially in CF patients, where treatments fail approximately half the time. To better reflect host conditions, media such as artificial sputum medium (ASM) and synthetic cystic fibrosis medium (SCFM-2) have been developed. We tested the susceptibility of *S. aureus* USA300, one of the most widely circulating *S. aureus* strains, to 9 representative antibiotics from different classes in MHB and ASM. Most significantly, USA300 is 128-fold more sensitive to cefuroxime, a β-lactam, in ASM relative to MHB. To investigate the mechanism for this altered response, we performed a genomic screen of a sequence defined transposon library of USA300 in the presence and absence of cefuroxime. The screen and subsequent phenotypic assays identified mutants harbouring mutations that cause disruptions broadly related to either the cell envelope and cellular responses. Mutants related to the cell envelope include those related to the peptidoglycan, wall teichoic acid (WTA), and lipoteichoic acid (LTA). The mutants related to cellular stress responses are those related to cell envelope stress response *graXRS vraFG* five component system and the stringent response. By investigating alterations to these systems, we unveiled remodelling of the MRSA USA300 cell envelope in CF-mimetic conditions, including decreased abundance of PBP2, 2a, 3, and 4 and WTA, decreased polymer length for WTA and LTA, and increased cell surface hydrophobicity. For stress responses, we identified a loss of antibiotic-mediated induction of GraXRS-VraFG and stringent response in CF media. Finally, we sought to further characterize alterations at the cell envelope using AFM. We found that USA300 cells grown in SCFM-2 had significant increase in surface roughness, cell diameter, and cell elasticity with a reduction in adhesion compared to those grown in MHB. This work provides insights into the discordance between standard AST results and therapeutic outcomes for *S. aureus* in CF-mimetic conditions. Our results demonstrate unexpected hyper susceptibility of USA300, a MRSA strain, to cefuroxime in ASM. Typically, β-lactams would not be recommended for usage against USA300 based on standard AST. However, our results indicate that β-lactams may be a viable choice in treating MRSA infections in CF patients. Overall, host-mimetic media, such as ASM, may serve as a better model for measuring bacterial resistance and correlate more strongly with positive clinical outcomes. Together, our AST results in CF-mimetic conditions provides insights into bacterial responses under these conditions, uncovers mechanistic details into alterations to unexpected sensitivity to β-lactams, and could guide and optimize the use of currently available antibiotics.

Gen abstract type

Poster

Section Type

I&I

40 - Streptococcal superantigen redundancy as a mechanism to evade humoral immunity

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Streptococcus pyogenes is a globally prominent human-specific pathogen. Though typically an asymptomatic nasal colonizer, *S. pyogenes* can cause mild illness such as pharyngitis to severe infections such as flesh-eating disease. *S. pyogenes* possesses a wide variety of virulence factors, including a family of potent exotoxins called 'superantigens' that are essential to the establishment of infection and disease progression. These toxins target two critical receptors of our adaptive immune system: the T cell receptor (TCR) and major histocompatibility class II (MHC-II) molecules. Superantigens crosslink TCRs with MHC-II molecules in a manner that bypasses the antigen specificity required for conventional T cell activation, resulting in the mass proliferation of T cells. To date, 16 genetically distinct streptococcal superantigens have been identified and individual strains of *S. pyogenes* encode unique repertoires thereof. Using a transgenic mouse model in which mice express human MHC-II, our lab previously established that *S. pyogenes* requires superantigens for productive nasopharyngeal infection and that vaccination with inactive superantigen generates a protective antibody response against the strain that produces it. Despite the importance of superantigens in *S. pyogenes* infection, it remains unknown why *S. pyogenes* encodes such an excess of these toxins. We hypothesize that *S. pyogenes* utilizes superantigen redundancy as a mechanism to evade host anti-superantigen immune responses. Three superantigens with strong, robust responses, SpeA, SpeH, and SmeZ are being used as model superantigens to test this. A toxoid SpeA vaccine has previously been shown to be protective against infection with the SpeA-producing strain MGAS8232, however this vaccine conferred no protection against the SpeH- and SmeZ-producing strain HKU16. A toxoid SpeH vaccine was not protective either and an HKU16 SpeH knockout strain confirmed that this superantigen is not required for infection with this strain, suggesting SmeZ likely contributes. These results demonstrate that anti-superantigen immunity is not protective across strains with unique superantigen repertoires nor is it effective if a strain expresses multiple active superantigens. Overall, knowledge of how superantigens may contribute to the evasion of immune responses will provide a greater understanding of *S. pyogenes* pathogenesis and is essential to evaluating superantigens as vaccine antigen candidates.

Gen abstract type

Poster

Section Type

I&I

50 - Antifungal resistance in Candida bloodstream infections: a global systematic review and meta-analysis

Yindi Xiong[1, 2, 3], Benjamin Caddey[1, 2, 3], Gilbert Lazarus[4], Claire Kamaliddin[2, 5], Herman Barkema[1, 2, 3], Raph Hampers[4, 6], John Conly[2, 3, 7]

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Candida bloodstream infections are a major cause of hospital-associated morbidity and mortality worldwide, but global antifungal resistance data remain fragmented and disproportionately derived from high-income settings. This limits understanding of where resistance is increasing, which species are driving change, and how patterns differ across regions and income settings. We aimed to estimate the global prevalence and temporal trends of antifungal resistance in *Candida* bloodstream infections, stratified by species, WHO region, and country-income level.

We conducted a systematic review and meta-analysis of peer-reviewed studies published between January 2010 and May 2024 that reported antifungal susceptibility testing results for *Candida* species isolated from blood cultures. MEDLINE, Embase, and Global Health were searched. Multilevel random-effects meta-analysis was used to estimate pooled resistance prevalence, and mixed-effects meta-regression was used to assess temporal trends.

A total of 322 studies from 33 countries, comprising 150,737 bloodstream isolates, were included. Overall, *Candida albicans* remained largely susceptible across antifungal classes. Global fluconazole resistance in *C. albicans* was 2.6% (95% CI 1.6-3.9), although resistance increased over time in Southeast Asia and in low- and middle-income countries (LMICs). In contrast, non-*albicans Candida* showed higher and more dynamic resistance patterns. *Candida glabrata* had the highest global fluconazole resistance at 14.0% (10.7-17.7), with substantial regional variation, including a marked increase in Southeast Asia but a decline in high-income settings. *Candida tropicalis* also showed high fluconazole resistance in Asia, particularly in Southeast Asia and the Western Pacific, but without a clear temporal increase. The clearest emerging signal was *Candida parapsilosis*, which showed global fluconazole resistance of 9.5% (6.4-13.2), higher resistance in LMICs than in high-income countries, and the steepest global increase over time at 1.41% annually. Echinocandin resistance remained generally low overall, although it remained clinically relevant in *C. glabrata*. Amphotericin B resistance was rare, but upward trends were still observed in selected strata.

Antifungal resistance in candidemia follows strongly species-specific trajectories and should not be interpreted as a single pattern across *Candida* species. The key global signal was not rising resistance in *C. albicans*, but increasing fluconazole resistance among non-*albicans Candida*, particularly *C. parapsilosis*. This pattern is concerning because it is consistent not only with antifungal selection pressure, but also with healthcare-associated transmission. These findings support the need for species-level diagnostics, routine susceptibility testing, and stronger fungal AMR surveillance, especially in LMICs where surveillance gaps remain substantial.

Gen abstract type Poster

Section Type I&I

54 - Phage cocktail therapy protects against APEC O78 infection and stimulates innate immune responses in poultry

Mawra Gohar[1], Jenny Hyun[1], Matthew Waldner[1], John Morris Fairbrother[2], Charles Martin Nyachoti[3], M. Faizal Abdul Careem[1], Dongyan Xu Niu[1]

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Avian pathogenic *Escherichia coli* (APEC), the causative agent of colibacillosis, is responsible for substantial economic losses in the poultry industry due to mortality, decreased egg production, and increased veterinary interventions. The growing prevalence of antimicrobial resistance (AMR) and restrictions on antibiotic use in food-producing animals have intensified the search for alternative disease control strategies. Bacteriophages (phages) represent a promising bactericidal approach; however, their *in vivo* efficacy in laying hens and host immune interactions remain incompletely understood. This study investigated (i) the efficacy of a multi-phage cocktail against APEC O78 infection in laying hens and (ii) the innate immune responses induced by individual phages in chicken macrophages.

Three lytic double-stranded DNA phages infecting APEC O78—ASO78A (*Tequatrovirus*), ASO78B (unclassified *Caudoviricetes*), and AVIO78A (*Sashavirus*)—were combined as a phage cocktail for *in vivo* evaluation. In a laying hen infection model, specific-pathogen-free hens (25-27 weeks old; n = 10 per treatment group) received the phage cocktail either through drinking water (DW) for four consecutive days or as a single intramuscular (IM) injection prior to intravenous challenge with APEC O78 (10^8 CFU). Phage cocktail treatment reduced mortality from 20% in the APEC-only group to 0% and decreased morbidity by approximately 30%. Birds receiving phages through DW showed a marginal trend toward reduced body-weight loss compared with IM-treated or untreated birds ($p = 0.063$). Macroscopic lesions were detected in 6.7-11.7% of examined organs in APEC-only and IM groups but were absent in DW-treated birds. Histopathological examination demonstrated significantly reduced lesion severity in DW-treated hens, with the most pronounced reduction observed in the spleen ($p = 0.006$). Phages were recovered from multiple organs, with concentrations ranging from 1.3 to 2.1 log10 PFU/g, demonstrating systemic distribution following administration. Importantly, 16S rRNA gene sequencing revealed that phage cocktail treatment did not significantly alter the overall structure or diversity of the cecal microbiota.

To evaluate host immune responses, the MQ-NCSU chicken macrophage cell line was exposed to individual phages (ASO78A, ASO78B, and AVIO78A) at multiplicities of infection (MOI) of 50 and 100. Individual phages significantly induced inducible nitric oxide synthase (iNOS) expression and nitric oxide production ($p < 0.0001$), indicating activation of macrophage antimicrobial functions. Pro-inflammatory cytokines, including interleukin-1β and interleukin-6, were upregulated ($p < 0.05$-0.0001), whereas tumor necrosis factor-α responses were modest and interleukin-10 induction was limited. Notably, ASO78A and ASO78B, but not AVIO78A, induced type I interferon responses (IFN-α and IFN-β; $p < 0.01$-0.0001), highlighting phage-specific differences in immunomodulatory potential. Pharmacological inhibition experiments demonstrated that iNOS/NO production was dependent on Toll-like receptor 4 (TLR4) and nuclear factor-κB signaling pathways, whereas type I interferon responses were only partially TLR4-dependent and largely independent of NF-κB activation.

Together, these findings demonstrate that phage cocktail therapy effectively controls APEC infection in laying hens without disrupting gut microbiota, while individual phages stimulate innate immune responses through distinct signaling pathways. This work highlights the dual antibacterial and immunomodulatory roles of phages and supports their application as sustainable alternatives for controlling colibacillosis in poultry production.

Abstract category choice

Poster

Section Type

I&I

55 - Defining the Molecular Basis of Nitrosative stress defense in Clostridioides difficile

Margaret Opeoluwa[1], Abiola Olaitan[1]

[1]University of Waterloo

Introduction: *Clostridioides difficile (C. difficile)* is a Gram-positive spore-forming, toxin-producing anaerobic bacteria. The pathogen is the leading cause of hospital-acquired diarrhea. In response to *C. difficile* infection (CDI), the host innate immune system activates immune cells that produce bactericidal reactive chemicals. These include reactive nitrogen species (RNS), such as nitric oxide (NO) and peroxynitrite ($ONOO^-$). Elevated levels of RNS induce nitrosative stress that damages bacterial macromolecules such as DNA and proteins, disrupting essential physiological process and ultimately leading to cell death. Despite exposure to host-derived RNS, *C. difficile* can persist within the gut. Therefore, it is evident that the bacterium has evolved mechanisms to withstand nitrosative stress. This study investigates the molecular mechanism underlying *C. difficile* adaptation to nitrosative stress and examines whether prior sublethal exposure induces a primed adaptive state.

Methods: The minimum inhibitory concentrations (MICs) of the RNS donors diethylamine NONOate (NO donor; DETA/NO) and $ONOO^-$ were determined. Next, the pathogen was subjected to RNS-dose dependent growth kinetics. Priming was performed by first exposing cells to a sublethal dose of NO (1/16× MIC) to induce a primed state, followed by challenge with lethal NO concentrations. The impact of nitrosative stress on toxin production was assessed using an enzyme-linked immunosorbent assay (ELISA). Transcriptomic response was analyzed via RNAseq. The role of the transcriptional regulator *hcpR* in the priming was assessed using transposon mutant and complemented strain. Finally, the persistence of the primed phenotype was assessed across generations without nitrosative stress.

Results: In this study, we show that *C. difficile* tolerates RNS donors with MICs ranging from 0.5 mM -1 mM and 2 mM for DETA/NO and $OONO^-$, respectively. *C. difficile* demonstrated dose-dependent tolerance to DETA/NO, but concentrations ≥ 0.5 mM were lethal, completely inhibiting growth, while 0.0625 mM supported growth comparable to the untreated control. Further, prior exposure to a sublethal concentration of 0.0625 mM DETA/NO primed *C. difficile*, allowing it to grow upon subsequent exposure to lethal NO concentrations of 0.5-1 mM. Exposure to 0.0625 mM led to 1.52-fold reduced toxin production compared to vehicle control, indicating that exposure to RNS negatively impacts toxin production in *C. difficile*. Transcriptomic analysis revealed that naive cells exposed to lethal NO concentration showed extensive gene expression changes, including activation of DNA damage and repair pathways. In contrast, primed cells exposed to lethal NO displayed profiles like naive cells under sublethal NO, indicating enhanced cellular fitness. The transcriptional regulator *hcpR*, which controls nitrosative stress defense genes including *hcp* and *frdX*, was upregulated under lethal NO. The *hcpR*::Tn mutant remained sensitive despite priming, whereas complementation restored growth to wild-type levels The primed state gradually declined and was completely lost after two generations without nitrosative stress.

Conclusion: *C. difficile* exhibits both innate and inducible (primed) resistance to nitrosative stress. Sublethal NO exposure induces a primed state that enhances survival under otherwise lethal concentrations. *hcpR* is essential for mediating the adaptive response, and primed state diminishes without stress. Future research will explore epigenetic mechanisms contributing to the maintenance and regulation of priming in *C. difficile*.

Gen abstract type Poster

Section Type I&I

81 - Structural basis for CesT-dependent recognition of type III secretion system effectors in Enteropathogenic *Escherichia coli*

Senthuran Mahendradeva[1], Rachel Bliss[1], Paniz Pour Abdi[1], Dustin Little[1]

[1]Toronto Metropolitan University

Enteropathogenic *Escherichia coli* (EPEC) is an enteric pathogen that infects humans through the oral-fecal route causing diarrheal disease. Its primary tool for infecting is a needle-like molecular structure termed the type III secretion system (T3SS), which releases toxin-like proteins termed effectors into the host cell (Deng et al. 2017). Interestingly, EPEC infection occurs in three stages. EPEC infection occurs in three stages: intimate attachment to the host cell, release of about 20 effectors that manipulate host signaling pathways to promote bacterial survival and replication, and adaptation to the host-bound state. While there is understanding of the stages, the field lacks structural data of the mechanism that drives interaction hierarchy by CesT and how effectors are prioritized over other CesT substrates inside the bacteria. To address this, we set out to determine the structural basis of Map and NleG interacting with CesT. We co-expressed CesT with Map or NleG, purified the protein complex and performed protein crystallization trials. Both protein complexes were crystallized, where Map-CesT complex X-ray diffracted to 2.5 Å, while NleG-CesT diffracted to 2.0 Å. In both structures, the CesT dimer was bound to two peptides of the effector. Additionally, both Map and NleG contained the conserved β-motif that is observed in T3SS chaperone-effector interactions. In addition, Map and NleG contain a downstream region that makes additional contacts with CesT. We termed the downstream region of Map and NleG as the α-helix motif as it is conserved in both effectors. We constructed glutamate mutants of these key hydrophobic residues and proved their ability to interact with CesT and their translocation level into the host cell. Disrupting residues of the β-motif and the α-helix motif disrupted interaction with CesT and translocation of the effectors into the host cell. Furthermore, structural alignment of Map and NleG fragments from our structure with the CesT-CsrA, shows overlapping binding interfaces . Specifically, there is interaction of the C-terminus and the α-helix motif of Map and NleG on the same concave surface of CesT. We generated a consensus sequence based on the α-helix motif of Map and NleG and searched against other effectors that depend on CesT and we identified multiple other effectors containing this motif. Together these data suggest a competitive binding of effectors to CesT to prevent CesT self-association that leads to CesT-CsrA complex, effectively preventing premature adaptation to the host-bound state without releasing effectors.

Gen abstract type Poster

Section Type I&I

82 - The antagonistic and synergistic effect of polyamines on polymyxin efficacy against clinical isolates of *K. pneumoniae* and *P. aeruginosa*

JOSHUA ADAMS[1], Omar El-Halfawy[1]

[1]University of Regina

The chemical landscape encountered by bacteria in their environment could shape their responses to different stress factors; these altered responses are not detected in standard lab conditions. Polyamines are a class of compounds that are present in the bacterial environment that have been shown to alter cellular stress responses in bacteria and are over produced in host organisms of pathogenic bacteria. At infection-relevant concentrations, polyamines have been shown in *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* to cause polymyxin (PMX) resistance —a last resort, cationic, membrane-acting antibiotic class— by localizing at the membrane and competing for cell surface binding. However, we recently identified a clinical isolate of *Klebsiella pneumoniae* where polyamines reverse PMX resistance. The prior reports on the protective effects of polyamines against PMX treatment focused on select prototypic strains and not on isolates from diverse bacterial backgrounds. Thus, we tested 200 clinical isolates of the World Health Organization's critical priority pathogens—*P. aeruginosa* and *K. pneumoniae*. We confirmed that polyamines can induce PMX resistance in certain isolates (antagonism) and we contrarily identified strains in which polyamines reverse PMX resistance (synergy) and strains that exhibit no change in PMX susceptibility (additive). To investigate why polyamines attenuate PMX resistance in certain isolates, we conducted two complementary genomic approaches utilizing comparative genomic analysis and chemogenomics. Comparative genomic analysis identified mutations in synergistic isolates previously shown to confer PMX resistance via upregulation of the *arnBCADTEF* operon. These observations were further supported by converting an antagonistic *P. aeruginosa* strain to a synergistic phenotype through introducing a mutation that upregulates this operon, suggesting polyamine-PMX synergy is in part, functionally linked to the upregulation of the *arnBCADTEF* operon. We conducted a chemogenomic screen using a sequence-defined mutant library of the non-essential genome of *K. pneumoniae* MKP103, a PMX-resistant strain due to an upregulated *arnBCADTEF* operon. The screen results and subsequent Cytochrome C binding assays implicate the alteration in surface charge in the polyamine-PMX synergy. Using a fluorescent derivative of PMX and membrane permeabilization assays we showed that pre-exposure of *K. pneumoniae* MKP103 to polyamines led to an increase in PMX surface binding and potentiated PMX membrane perturbation. Both of these effects were absent in *K. pneumoniae* antagonistic isolates. Conversely, simultaneous exposure to polyamines and PMX reduced PMX surface binding in *K. pneumoniae* antagonistic isolates, consistent with previous observations in *Burkholderia cenocepacia* which partly explained the polyamine-PMX antagonism. However, simultaneous exposure of PMX and polyamines on synergistic isolates had no effect on PMX binding. Thus, we propose that polyamines suppress surface charge alterations associated with PMX resistance, thereby enabling membrane permeabilization and synergy with PMX. This work expands our understanding of bacterial responses to PMX under conditions that more closely reflect the infection environment. Specifically, we build on the established role of polyamines in protecting bacteria from PMX to show that, paradoxically, polyamines can synergize with PMX in resistant clinical isolates. These findings may inform novel strategies to combat PMX-resistant infections.

Gen abstract type Poster

Section Type I&I

102 - Development of a Multiplex Epoxy-Slide Antigen Microarray for IgG Subclass Characterization of Conserved Group A *Streptococcus* Vaccine Antigen

Tania Nasreen[1], Gregory J. Tyrrell[1, 2]

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Background: Group A *Streptococcus* (GAS) remains a major cause of pharyngitis, invasive infections, and immune-mediated sequelae worldwide. Conserved epitope-based vaccine candidates derived from the M protein C-repeat region (J8, p*17) and the SpyCEP virulence factor (K4S2) have progressed to early-phase clinical evaluation. While total IgG responses are commonly assessed in vaccine studies, IgG subclass profiling can provide additional insight into functional immune mechanisms, particularly complement activation and opsonophagocytic activity. To address the need for scalable and cost-efficient subclass analysis, we propose the development of a multiplex epoxy-slide antigen microarray targeting conserved GAS vaccine antigens.

Objective and Hypothesis: We aim to develop a cost-efficient multiplex epoxy-slide antigen microarray capable of quantifying total IgG and IgG subclasses (IgG1 and IgG3) against conserved GAS vaccine antigens in longitudinal human serum samples. We hypothesize that this platform will sensitively detect vaccine-induced increases in antigen-specific IgG and reveal subclass-specific response patterns that are differentially associated with Fc-mediated effector function. In particular, antibodies targeting surface-associated M protein epitopes (J8 and p*17) and the SpyCEP-derived K4S2 epitope are expected to elicit both IgG1 and IgG3 responses, with IgG3 potentially contributing disproportionately to opsonophagocytic activity despite lower relative abundance.

Methods: Core peptide antigens J8, p*17, and K4S2, together with recombinant CRM197, will be covalently immobilized onto epoxy-coated glass slides via primary amine coupling. Antigens will be printed in replicate arrays at two concentrations to assess assay dynamic range and reproducibility. Two concentrations help ensure the assay can accurately detect both low and high antibody responses while maintaining quantitative measurement within the linear detection range. Slides will be blocked using a casein-based buffer and incubated with diluted human serum samples. Detection will be performed using far-red fluorophore-conjugated secondary antibodies specific for total IgG, IgG1, and IgG3. Slides will be imaged using a gel documentation system optimized for far-red fluorescence. Quantitative analysis will include background subtraction, replicate averaging, and coefficient-of-variation (CV) assessment to evaluate assay reproducibility.

Anticipated Results: We anticipate that the assay will detect increased antigen-specific total IgG responses following vaccination relative to baseline samples. Antigen-specific responses to J8, p*17, and K4S2 are expected to include both IgG1 and IgG3 subclasses, with potential variability in subclass distribution across individuals and antigens. Assay reproducibility will be evaluated using replicate antigen spots, with acceptable precision defined as a coefficient of variation (CV) <15%. The platform is therefore expected to demonstrate low background fluorescence, adequate dynamic range, and sufficient sensitivity to discriminate longitudinal antibody responses across timepoints.

Conclusion: This multiplex epoxy-slide antigen microarray is expected to provide a scalable and economical platform for detailed characterization of GAS vaccine-induced humoral immunity. To our knowledge, this represents one of the first applications of an epoxy-slide-based multiplex microarray for IgG subclass profiling of conserved GAS vaccine antigens, offering a practical and cost-effective alternative to bead-based immunoassays. IgG subclass profiling may enhance mechanistic interpretation of antigen-specific responses and facilitate scalable immune monitoring in future GAS vaccine studies.

Gen abstract type

Poster

Section Type

I&I

113 - Mapping the physical and genetic interactome of *Pseudomonas syringae* effectors

Janis H. T. Cheng[1], Bradley Laflamme[2], John Macpherson[2], Charles Boone[2], Alexander W. Ensminger[2], Darrell Desveaux[1], David S. Guttman[1]

[1]Cell & Systems Biology, University of Toronto, [2]Department of Molecular Genetics, University of Toronto

Many bacterial pathogens employ a type III secretion system to deliver effector proteins into host cells to promote virulence. One of the best-studied collections of type III secreted effectors (T3Es) belongs to the phytopathogen *Pseudomonas syringae*, which can infect a broad range of agriculturally significant crop species. The dynamic relationships between pathogen effectors have been shown to dramatically influence the outcome of plant-microbe interactions. Despite the importance of effector coordination in modulating plant immune responses and pathogen fitness, few have systematically studied this phenomenon. Here, I describe a study to comprehensively and systematically map all genetic and physical interactions between a library of ~500 effectors representing the pangenome *P. syringae* effector repertoire. Using high-throughput yeast genetic screens, our work has interrogated >270,000 pairwise effector combinations, covering the entire effector-effector interaction space across the *P. syringae* species complex. We constructed a network representing binary physical/ genetic interactions, revealing patterns of specificity in effector-pairing and complexity in effector repertoire composition. This data provides testable hypotheses regarding mechanisms underlying infection strategies and effector regulatory relationships that play central roles in virulence.

Gen abstract type

Poster

Section Type

I&I

118 - Bioinformatic and genomic investigation of the AdeQ-AdeDE efflux pump in *Acinetobacter pittii*

Elaine Schultz[1], Ayush Kumar[1]

[1]University of Manitoba

Acinetobacter species are Gram-negative opportunistic pathogens primarily causing nosocomial infections. A very important tool employed by these species for antimicrobial resistance are the resistance-nodulation division (RND) efflux pumps. The most well-known pumps from *Acinetobacter* spp. are AdeAB(C), AdeIJK, and AdeFGH, each consisting of a known regulator and three parts: the outer membrane factor, the periplasmic adapter protein, and the RND-transporter. The AdeDE RND-efflux pump was first identified in *A. pittii* when it was known as “genomic species 3”, where a hypothetical protein when translated showed 50% amino acid identity to AdeB and so was designated AdeE, an RND transporter. The outer membrane factor for AdeDE remains unknown, but a potential TetR-like transcriptional regulator has been identified immediately upstream of the *adeDE* operon and designated as *adeQ*.

Past studies suggest that the presence of AdeDE pump only in *A. pittii*. Using bioinformatics analysis, we studied the prevalence of AdeDE pump in other *Acinetobacter* species. Upon a BLASTn search, selection of 13 whole genome sequences of *Acinetobacter* species containing *adeQDE* were aligned. We detected the presence of AdeDE and AdeQ genes in *A. nosocomialis, A. lactucae, A. baumannii, A. calcoaceticus* and *A. seifertii*. This indicates that the AdeDE pump is more prevalent in *Acinetobacter* spp. than previously thought. This comparison revealed high conservation, not only at the nucleotide level of *adeQDE*, but also of genes surrounding *adeQDE*, specifically 4 genes upstream and 14 gene downstream. Insertion elements as well as genomic island evidence was found around these genes. From this evidence, it implies that horizontal gene transfer is a possibility of how different *Acinetobacter* spp. are obtaining these genes.

To determine the role of AdeQ in the regulation of *adeDE*, we deleted *adeQ* in an *A. pittii* strain. Deletion of the *adeQ* resulted in an increase in *adeD*, suggesting that AdeQ is a repressor of *adeDE*. Curiously, cultures of the *A. pittii adeQ* deletion strain exhibited a severe growth defect when grown in LB media and cellular abnormalities in the form of clumps were observed using scanning electron microscopy. Despite the increase seen in *adeD* expression, there was no real impact in the ethidium bromide efflux nor in minimum inhibitory concentrations.

Our work shows that the AdeDE pump is more prevalence among *Acinetobacter* spp. than previously thought. Further, our data suggest that AdeQ represses the expression of AdeDE. However, the role of AdeDE in antibiotic susceptibility of *A. pittii* needs further investigation.

Gen abstract type

Poster

Section Type

I&I

124 - Characterization of antimicrobial resistance in neglected bacterial agents of bovine respiratory disease complex

Sara Andres-Lasheras[1], Chloe Brennan[1], Uvindu Abeysekara[1], Sujeema Abeysekara[1], Sheryl Gow[2], Rahat Zaheer[1], Tim McAllister[1]

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Bovine respiratory disease (BRD) is the most significant and costly disease in North American feedlot cattle. Antimicrobials are the mainstay for preventing, treating, and controlling BRD, but their administration can select for antimicrobial resistance (AMR). Integrative and conjugative elements (ICE) are mobile genetic elements (MGE) capable of horizontal transfer between bacteria. ICE have been detected in BRD bacteria isolated from both healthy and BRD-sick cattle with some ICE harboring up to 12 antimicrobial resistance genes (ARG) coding for resistance to 7 antimicrobial classes. While AMR in classical BRD pathogens has been extensively studied, *Trueperella pyogenes* (Tp) and *Bibersteinia trehalosi* (Bt) remain largely neglected, leaving a critical knowledge gap regarding their prevalence, potential involvement in BRD, and contribution to the respiratory AMR reservoir in beef cattle. Emerging evidence suggests that Tp and Bt may harbor MGE/ICE-ARGs but their epidemiological relevance and AMR dynamics remains largely unexplored. Additionally, *Mycoplasma bovis* (Mb) is often overlooked in AMR surveillance, despite its documented involvement in BRD and increasing resistance profiles. This study aims to address these knowledge gaps by characterizing phenotypic and genotypic AMR in Bt, Tp, and Mb isolated from western Canadian feedlot cattle.

Respiratory samples were collected from clinically healthy and BRD-affected calves at feedlot entry and during the feeding period, between 2014-2026, from Alberta, Saskatchewan, and Ontario. *In vitro* antimicrobial susceptibility testing was carried out for Bt (n = 86), Tp (n = 219), and Mb (n = 447) isolates using the microdilution method following CLSI guidelines when available.

The recovery of Bt (0.7 - 1%), Tp (0.4 - 2.4%), and Mb (16.1%) from healthy calves was consistently lower than from BRD calves (9.3%, 13.5 - 28.3%, and 22.3%, respectively). Bt and Tp isolates from sick calves showed overall higher AMR levels compared with isolates from healthy animals. Mb isolated from healthy calves showed high AMR towards macrolides and low AMR for enrofloxacin. Mb isolates carrying putatively functional ICE had higher AMR levels compared to isolates lacking them.

Although less frequently detected in healthy feedlot cattle, Bt and Tp were more prevalent in BRD-affected calves, suggesting their potential role in BRD and reduced susceptibility to antimicrobials commonly used for treatment. MGE like ICE may also play a role in the horizontal transmission of AMR in Mb. Ongoing whole genome sequencing of Bt and Tp will investigate the presence of MGE/ ICE-ARG in order to provide a more complete understanding of the AMR reservoir array within these BRD-associated bacteria.

Gen abstract type

Poster

Section Type

I&I

131 - Exploring the pathogenic potential of the bacterial genus *Pantoea* using the Galleria *Mellonella* infection model

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[1]The University of Regina

Pantoea is a diverse genus of Gram-negative bacteria found in association with plants, insects, and animals. While members of this genus are generally isolated from the environment, isolates have also been identified in nosocomial settings, contaminating hospital equipment and causing opportunistic infections in humans that include septic arthritis, synovitis, and soft-tissue infections. Although *Pantoea* is considered an opportunistic pathogen, little is known about its pathogenicity and virulence in non-plant hosts. In this study, our objective was to evaluate the pathogenic potential of several *Pantoea* species using the *Galleria mellonella* infection model. Infection assays were performed using 15 strains of *Pantoea* within five species. *G. mellonella* larvae were inoculated with each strain via intra-hemocoelic injection and were monitored for survival and health status at 24-hour intervals for 72 hours. Kaplan-meier curves were plotted to compare virulence between strains. Nine strains were identified as being virulent relative to controls. *P. stewartii* 626 exhibited the highest virulence, having a percent survival of 30.6% at 72 hours post-injection. Despite being the most virulent of the *Pantoea* strains, *P. stewartii* 626 was less virulent than the human pathogen *A. baumannii*, with the latter having a percent survival of only 7.2% at 72 hours post-injection. In contrast to 626, the eight other virulent *Pantoea* strains demonstrated significantly lower virulence with percent survival values ranging between 50.4% and 81.5% at 72 hours post-infection. The other *Pantoea* strains tested were identified as being non-virulent, with survival curves concordant to that of BL21. There was no significant correlation between species nor source of isolation and virulence, indicating that pathogenic potential may be strain-specific. Exploration of virulence in *Pantoea*, and in particular the genetic determinants that enable host-association, will enhance our understanding of how environmental bacteria adapt to new hosts, and will provide insight into opportunistic infections in humans.

Gen abstract type

Poster

Section Type

I&I

135 - Drug hypersensitivity in vancomycin-intermediate *S. aureus* facilitates eradication in a macrophage infection model and limits resistance *in vitro*

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Vancomycin has long served as a 'last-resort' antibiotic for infections unresponsive to other treatments. However, growing resistance in *Staphylococcus aureus* increasingly undermines its effectiveness. Vancomycin-intermediate *S. aureus* (VISA) represents a key threat to effective vancomycin therapy, as these strains require higher vancomycin concentrations for inhibition, often exceeding clinically achievable levels. This intermediate resistance not only complicates treatment outcomes but also signals adaptation toward full resistance. VISA infections are associated with prolonged illness, increased treatment failure, and higher healthcare costs, highlighting the importance of developing strategies to control VISA-associated infections. VISA often arises through mutations in the essential two-component system WalKR, leading to a thickened peptidoglycan layer that sequesters vancomycin and reduces susceptibility. Our lab has discovered that WalKR mutants underlying a vancomycin-intermediate phenotype exhibit markedly increased sensitivity to the nucleoside antibiotic tunicamycin. This phenomenon, termed collateral sensitivity, represents a rare evolutionary trade-off, in which resistance to one antibiotic confers hypersensitivity to another. We found that this phenotype emerges consistently and demonstrated that exploiting this hypersensitivity enhances clearance of VISA mutants in a macrophage infection model—the first known case where collateral sensitivity has been shown to improve clearance *in vivo*. Furthermore, by combining vancomycin with a tunicamycin dose 64-fold lower than its inhibitory concentration, we were able to inhibit the evolution of vancomycin resistance during experimental evolution. Overall, this work lays the foundation for leveraging collateral sensitivity to improve infection clearance and impede resistance evolution.

Gen abstract type

Poster

Section Type

I&I

138 - Characterizing the Cyanide-Insensitive Oxidase of *Pseudomonas aeruginosa*

Natascia Ciancibello[1], Camille Vasseur[1], Sandaru Ileperuma[1], Justin Di Trani[1]

[1]University of Alberta

Pseudomonas aeruginosa (PA) is a highly antibiotic-resistant bacterium that has been repeatedly declared a critical threat to public health by the World Health Organization and contributes to significant infection rates in individuals with cystic fibrosis. Understanding how PA colonizes the body is essential for developing therapeutics to counter its mechanisms of resistance, which rely heavily on quorum sensing to enable cell-to-cell communication and rapid adaptation. Quorum sensing is intrinsically tied to the electron transport chain (ETC), and, more specifically, to a unique terminal oxidase called the cyanide-insensitive oxidase (CIO), which allows PA to grow in the presence of the cyanide it secretes to kill other cells and regulate spontaneous PA mutants, as well as resist macrophages. We have determined the first bacterial CIO structure at 3 Å resolution using cryogenic electron microscopy (cryo-EM), revealing structural insights into how this class of enzymes differs from closely related oxidases. We have developed a high-throughput assay and screened the Enamine Bioreference Library of compounds to identify potential small-molecule CIO inhibitors and, consequently, interfere with PA's ability to withstand cyanide. Using cryo-EM and enzymatic assays, we will obtain high-resolution information on how the confirmed inhibitors bind and their affinities, enabling the rational design of improved inhibitors. Targeting this enzyme offers a promising avenue for developing novel therapeutics against multidrug-resistant PA and will provide broader insight into the ETC's role in PA pathogenicity.

Gen abstract type

Poster

Section Type

I&I

140 - Detection of antimicrobial resistance determinants in *Trueperella pyogenes* from British Columbia, Canada

Parichay Raj Subedi[1], Kazal Ghosh[1,2], Florence Huby[1], Joseph Elliot Rubin[1]

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Trueperella pyogenes is a Gram-positive bacterium that colonizes the skin and mucus membrane of the upper respiratory, gastrointestinal, and urogenital tracts of animals. As an opportunistic pathogen, this organism is associated with purulent (pus-forming) infections of the respiratory, digestive, and reproductive systems. These infections result in economic losses due to decreased milk production and quality, reproductive failures, and partial or total carcass condemnation during slaughter. Despite its clinical importance and frequent isolation from clinical specimens, there is limited information on its antimicrobial resistance (AMR) patterns. Furthermore, standardized interpretive criteria for categorizing an isolate as susceptible or resistant based on a susceptibility test are only available for four drugs. In this study, 116 *T. pyogenes* isolates grown from diagnostic submissions to the Animal Health Centre in British Columbia between 2023 and 2025 were sequenced to identify resistance determinants. Whole genome sequencing was performed using the Illumina platform. Genomes were assembled with Unicycler, and resistance genes were identified using ResFinder. Overall, resistance genes were detected in 68/116 isolates. The *tet*(W) gene was most commonly identified, present in 56.9% of isolates (66/116). This gene encodes a ribosomal protection protein conferring tetracycline resistance. Another tetracycline resistance gene, *tet*(33), was detected in 4.3% of isolates (5/116), all of which also harbored *tet*(W). The macrolide-lincosamide resistance gene *erm*(X) was found in 13.7% of isolates (16/116), the majority of which (14/16) also carried *tet*(W). The aminoglycoside and sulfonamide resistance genes *aad*A9 and *sul*1, respectively, were identified in 7.7% of isolates (9/116), always occurring together and with *tet*(W). Similarly, the aminoglycoside resistance genes *aph*(6)-Id*,* *aph*(3')-Ia*,* *aph*(3")-Ib*,* and chloramphenicol resistance gene *cmx* were identified together, and also with *tet*(W) in 6% of isolates (7/116). Genotypes suggesting multidrug resistance were identified in 16 isolates; genes associated with tetracycline, macrolide, aminoglycoside, phenicol, and sulfonamide classes were found. Co-location of genes conferring resistance to multiple classes within an isolate highlights the potential for the co-selection of resistance when antimicrobials are used. Furthermore, as some of these drug classes (particularly macrolides) are commonly used to treat infections in agricultural animals, this resistance has the potential to have a real-world impact on animal health. This study provides the first regional data on AMR determinants in *T. pyogenes* from British Columbia. The next step of this investigation will be to integrate genotypic and phenotypic data to correlate gene presence with categorical susceptibility. This study will provide useful context for interpreting the minimum inhibitory concentration (MIC), especially for the majority of drugs where standardized breakpoints are unavailable.

Gen abstract type

Poster

Section Type

I&I

169 - Isolation and Characterization of Bacteriophages Targeting Uropathogenic *E. coli*

Elodie Jolicoeur[1], Annie Chénard[1], Serena Tuytschaevers[1], Ally Champoux[1], Paul Sebastien Ugalde Silva[1], Louis-Charles Fortier[1]

[1]Department of Microbiology and Infectious Diseases, University of Sherbrooke

Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, with approximately 404.6 million cases reported annually and an incidence that continues to rise. Uropathogenic *Escherichia coli* (UPEC) is the primary causative agent, accounting for 65-75% of cases. The increasing prevalence of antibiotic resistance in *E. coli* significantly limits treatment options. In addition, the use of broad-spectrum antibiotics can disrupt the intestinal microbiota, potentially leading to dysbiosis and secondary complications. These limitations highlight the need for alternative antimicrobial approaches that selectively target pathogenic bacteria while preserving beneficial microbial communities. Bacteriophages (phages), viruses that infect and kill bacteria through highly specific interactions with bacterial surface receptors, represent a promising alternative. However, their narrow host range and the rapid emergence of resistance necessitate their use in cocktails composed of multiple phages. The design of such cocktails remains challenging, as interactions between phages can lead to synergistic or antagonistic effects, ultimately influencing therapeutic efficacy. A systematic evaluation of phage-phage and phage-host interactions is therefore essential for designing effective phage combinations.

The objective of this project is to isolate and characterize bacteriophages targeting UPEC strains and to evaluate their antibacterial efficacy individually and in defined combinations. Eighteen phages were isolated from environmental sources, including wastewater, swamps, and rivers, using three *E. coli* host strains. To ensure suitability for therapeutic applications, whole-genome sequencing was performed using Oxford Nanopore Technologies to identify lysogeny-associated genes and detect potential contamination. The assembled genomes were analysed using Pharokka and Phold for functional annotation, while vConTACT2 was used to cluster phages sharing significant gene content. Among the 18 phage lysates analyzed, three were found to contain temperate phages originating from the bacterial host chromosomes. These prophages likely excised from the host chromosome during lytic phage amplification, underscoring the importance of genomic characterization after amplification and cocktail preparation to ensure lysate purity and prevent the inadvertent administration of temperate phages. Among the 15 purified phage lysates, seven genetically distinct clusters were identified, revealing genomic diversity among the isolated phages. The genomic classification and host range analysis provided a rational framework for cocktail design, enabling the selection of phages from distinct groups to maximize diversity and reduce potential redundancy or competition. Subsequently, multiple phage combinations were evaluated in vitro to assess their collective lytic activity and ability to limit the emergence of bacterial resistance. The centroid index was used as a quantitative measure of infection efficiency and resistance suppression. Certain phage combinations exhibited reduced efficacy, consistent with antagonistic interactions, potentially due to competition for the same bacterial receptor. In contrast, other combinations displayed synergistic effects and suppressed the emergence of resistant bacterial populations.

Overall, this work enhances our understanding of phage-phage and phage-host interactions and supports the rational design of phage cocktails. Future work will focus on evaluating the most promising phage combinations *in vivo*, as well as assessing their stability, dosing strategies, and efficacy across a broader diversity of UPEC strains.

Gen abstract type Poster

Section Type I&I

172 - Prevalence and Spread of Antimicrobial Resistance in Bovine Respiratory Disease Pathogens Focusing on Cattle Morbidities and Mortalities

Chloe Brennan[1, 2], Sara Andres-Lasheras[2], Sheryl Gow[3], Athanasios Zovoilis[4], Karen Schwartzkopf-Genswein[2], Brian Warr[5], Steve Hendrick[6], Kim Stanford[1], Rahat Zaheer[2], Tim McAllister[2]

[1]University of Lethbridge, [2]Agriculture Agri-Food Canada, [3]Public Health Agency of Canada, [4]University of Manitoba, [5]Veterinary Agri-Health Services, Rocky View County, [6]Coaldale Veterinary Clinic

Bovine respiratory disease (BRD) is a multifactorial disease in beef and dairy cattle that significantly impacts the North American feedlot industry and is exacerbated by the emergence of antimicrobial resistance (AMR) in BRD-associated pathogens. BRD is primarily a polymicrobial infection involving *Pasteurella multocida (Pm)*, *Mannheimia haemolytica (Mh)*, and *Histophilus somni (Hs)*. These bacteria, typically commensals in the bovine nasopharyngeal microbiota, transition to pathogenic roles in stressed or immunocompromised hosts, resulting in acute or chronic respiratory disease. Notably, these pathogens carry antimicrobial resistance genes (ARGs) on mobile genetic elements (MGEs), particularly integrative and conjugative elements (ICEs). This study aims to investigate the prevalence of these BRD pathogens, their AMR phenotypes and genotypes, and their roles in disseminating AMR.

Nasopharyngeal swabs are being collected over a two-year period from feedlots across Southern Alberta from low- and high-risk cattle, clinically healthy animals and BRD-associated mortalities. Lung tissue samples were obtained exclusively from BRD-associated mortalities when possible. Detailed antimicrobial use metadata, including administration route, quantity, and purpose, were documented. Nasopharyngeal and lung samples were cultured on tryptic soy agar (TSA) media with sheep blood, with and without selective tetracycline or erythromycin. Presumptive isolates were confirmed via species-specific PCR and stored at -80°C for downstream phenotypic profiling through antimicrobial susceptibility testing and genomic characterization of ARGs and ICEs through whole genome sequencing.

To date, ~ 400 samples have been collected and processed with recovery of BRD bacteria as follows: *Mh*, 17%; *Pm*, 49%; and *Hs*, 32%. Antimicrobial susceptibility testing of these was carried out using broth microdilution methodologies in accordance with the Clinical Laboratory Standards Institute guidelines. Preliminary antimicrobial susceptibility results from morbidities and mortalities, show increased multi-drug resistance in AMR profiles compared to healthy controls. Increased similarity in AMR profiles among isolates across BRD-associated bacterial species further suggests the potential contribution of horizontal gene transfer to AMR dissemination.

This project will provide critical insights into the epidemiology, genomic composition, and dissemination mechanisms of ICEs in BRD pathogens, informing strategies to mitigate AMR in the feedlot industry.

Gen abstract type Poster

Section Type I&I

201 - Determining the effect of human serum on *Pseudomonas aeruginosa* physiology

Caroline Cui[1], Nicole Jervis[1], Matthew Chu[1], Joe Harrison[1]

[1]University of Calgary

The opportunistic bacteria *Pseudomonas aeruginosa (P. aeruginosa)* causes severe infections that are difficult to treat due to its intrinsic high antimicrobial resistance. Research and development into new treatments often start with *in vitro* antimicrobial assays traditionally performed in environments that are not an accurate reflection of the *in vivo* wound environment. For example, unpublished data from our lab suggests that the effectiveness of antimicrobial silver in clinical trials is surprisingly low despite its routine use for wounds in Canada and high antimicrobial success *in vitro*. Recent studies have started to use more biologically relevant environments by adding serum. Serum is the part of blood without red blood cells or clotting factors, and can be divided into complement (part of the body's innate immune defenses) and non-complement. While the effects of complement on *P. aeruginosa* have been investigated extensively, little to no research exists on the effects of complement-inactivated serum which contains over 3000 proteins. My work aims to address this knowledge gap. Our research shows that complement-inactivated serum elicits antimicrobial resistance and tolerance from *P. aeruginosa. P. aeruginosa* displayed tolerance to imipenem and colistin, and resistance to silver, when incubated with 10% complement-inactivated human serum. Through bioactivity-guided fractionation, we found a >100 kDa component in serum that elicits strong silver resistance and a component between 50-100 kDa that elicits weak tolerance and resistance. The addition of proteinase K, an enzyme that digests proteins, to the >100 kDa component resulted in a loss of silver resistance which suggests that this component is a protein. *P. aeruginosa* is also known to exhibit nutrition-dependent phenotypes, like virulence. We added proteinase K-digested human serum that was <50 kDa to provide the bacteria with an alternate nutrition source which elicited intermediate *P. aeruginosa* silver resistance. My research shows that bioactive moieties, chemistry, and physiology likely all play a part in serum-dependent antimicrobial resistance and tolerance. Further research will examine how complement-inactivated serum affects *P. aeruginosa* physiology through RNA-sequencing, which allows me to assess what parts of the DNA have been translated into RNA. Outputs will inform further research into creating better wound environment models that will provide the basis for *in vitro* antimicrobial R&D and wound-related research.

Gen abstract type

Poster

Section Type

I&I

208 - Metabolites produced by ESKAPE pathogens modulate the growth dynamics of *Acinetobacter baumannii*

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Acinetobacter baumannii is a Gram-negative, non-motile coccobacillus that has drawn increasing attention in recent years, particularly since its designation as a critical-priority pathogen in 2017. Its importance is further highlighted by its inclusion in the ESKAPE group—*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* spp.—a set of pathogens known for their ability to evade antimicrobial treatments and persist in clinical environments. These bacteria share traits that make them particularly successful in healthcare settings, including strong environmental resilience, diverse resistance mechanisms, and the global spread of high-risk epidemic clones.

In recent years, ESKAPE pathogens have increasingly been found together in co-infections, especially in immunocompromised patients. These infections often lead to more severe outcomes and complicate treatment strategies. Despite their clinical relevance, we still know relatively little about how these pathogens interact with one another and how these interactions influence their pathogenic potential. Understanding these relationships is essential for improving our ability to manage multidrug-resistant co-infections.

This project focuses on characterizing how *A. baumannii* is affected by its coexistence with other ESKAPE pathogens and their secreted products. Previous studies have shown that interspecies interactions can significantly alter bacterial behavior. For example, metabolites from *S. aureus* can promote the growth of P*. aeruginosa*, while metabolites from *P. aeruginosa* inhibit *Burkholderia cenocepacia*. More recently, phenol-soluble modulins (PSMs) produced by *S. aureus* have been identified as strong inhibitors of *A. baumannii* growth. In addition to secreted metabolites, many ESKAPE pathogens possess specialized secretion systems that contribute to interbacterial competition; *K. pneumoniae*, for instance, uses the Type VI Secretion System (T6SS) to kill neighboring cells.

To expand on these findings, growth-curve analyses and killing assays using cell-free supernatants were performed to determine how metabolites from each ESKAPE pathogen influence *A. baumannii* growth and behaviour. Our preliminary data show that supernatants from *S. aureus* initially inhibit *A. baumannii* growth, after which the bacterium recovers, resulting in a prolonged lag phase. Trypsin treatment of the supernatants eliminates this inhibitory effect, whereas heat treatment does not, suggesting that the active factor is a heat-stable, proteinaceous compound. Although the mechanism underlying the recovery of *A. baumannii* remains unclear, we suspect it involves metabolic or transcriptional adaptation. Supernatants from *P. aeruginosa* also reduce *A. baumannii* growth, but the factors responsible remain unidentified.

Moving forward, metabolomic analyses of the cell-free supernatants will be conducted to identify the compounds that may drive these inhibitory effects. Once top candidate metabolites are identified, we will expose *A. baumannii* to the purified compounds to validate the observed phenotypes, followed by generating targeted knockouts in the donor species to confirm their roles and further assess how they influence the growth dynamics of *A. baumannii*. We also aim to include the remaining ESKAPE pathogens to build a more complete picture of how *A. baumannii* behaves in polymicrobial environments. Finally, we will investigate whether exposure to these supernatants alters the sensitivity of *A. baumannii* to antibiotics or antiseptics, potentially revealing additional layers of interaction-driven modulation of its pathogenicity.

Gen abstract type Poster **Section Type** I&I

215 - Characterization of the Type 7 Secretion System in *Mycobacterium abscessus*

Shweta Parmar[1], Dr. Elitza Tocheva[1]

[1]University of British Columbia

Mycobacterium abscessus (Mab) is a rapidly growing non-tuberculous mycobacterium (NTM) and an emerging opportunistic pathogen. It is associated with severe pulmonary infections, particularly in individuals with underlying respiratory disorders such as cystic fibrosis. Its clinical management is complicated by an exceptionally high intrinsic resistance to antibiotics, often leading to persistent infections. Despite this, the molecular mechanisms that enable *Mab* to adapt and survive within host cells remain poorly understood. A key feature of mycobacterial pathogenesis lies in the Type VII secretion systems (T7SS), which are specialized membrane complexes that secrete effector proteins involved in nutrient acquisition, immune evasion, and virulence. Across mycobacteria, these systems have diversified into five subtypes (ESX-1 to ESX-5), each associated with distinct functions. However, unlike more extensively studied species such as *Mycobacterium tuberculosis*, that encodes the full repertoire of T7SS, *Mab* possesses only two systems: ESX-3 and ESX-4. Despite this reduced repertoire, *Mab* remains highly pathogenic and can drive severe secondary infections, suggesting that these systems may operate in a more streamlined yet highly efficient manner to support intracellular survival. While ESX-3 has been broadly linked to nutrient acquisition, the role of ESX-4 in *Mab* remains relatively undefined.

In this study, we combine advanced imaging approaches with infection-based analyses to investigate how ESX-3 and ESX-4 are localized within the mycobacterial cell envelope and explore their functional roles during infection of human macrophages. Using super-resolution fluorescent microscopy, we examine the spatial organization of T7SS components in intact *Mab* cells, revealing that these systems exhibit distinct localization patterns. Infection assays define the roles of these systems in intracellular growth, phagosome maturation and host inflammatory responses. Further, to connect structure with function, we employ cryo-electron tomography-based approaches that enable visualization of complex membrane structures and intracellular features in their near-native state. By positioning ESX systems at the center of *Mab* pathogenicity, this study provides insights into how rapidly growing NTMs coordinate infection and highlights T7SS as a promising avenue for future therapeutic interventions.

Gen abstract type

Poster

Section Type

I&I

227 - Investigating unconventional T cells as superantigen targets in menstrual toxic shock syndrome

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The most infamous pathology attributed to vaginal *Staphylococcus aureus* is the life-threatening disease known as menstrual toxic shock syndrome (mTSS). *S. aureus* encodes 26 known superantigens that mass-activate T cells in a Vβ-specific manner, bypassing the conventional peptide-specific interactions with antigen presenting cells. Interestingly, the superantigen TSST-1 is responsible for approximately half of all non-menstrual staphylococcal TSS cases, and is the sole causative superantigen behind mTSS, yet the reason why is unknown. Moreover, TSST-1 is the only staphylococcal superantigen which can target T cells containing Vβ2 receptors, which are disproportionately abundant among Mucosal Associated Invariant T (MAIT) cells. As MAIT cells are present in the endometrium and vagina, we hypothesize that TSST-1 specifically activates Vβ2 MAIT cells, driving the establishment of mTSS. In our work we use healthy human T cells isolated from peripheral blood in flow cytometry experiments. We have identified that purified TSST-1 triggers expression of the activation marker CD69 from ~20% of conventional T cells, yet upwards of 80% of MAIT cells. In contrast, purified TSST-1 with a mutation impairing TCR binding (S72A) initiates significantly less activation. Additionally, exposure to a *S. aureus* strain where we have deleted the TSST-1 gene (Δ*tst*) abolishes the pro-inflammatory cytokine response from Vβ2 MAIT cells relative to the wild-type strain. These findings strongly suggest that MAIT cells are more sensitive to TSST-1 than typical T cells, and there is targeted activation through the Vβ2 T cell receptor. Finally, depleting MAIT cells results in significantly less activation of Vβ2 T cells by purified TSST-1, highlighting the importance of MAIT cells in establishing a robust activation response. Overall, this work provides us with essential knowledge about host-pathogen interactions and MAIT cells in the context of menstrual immunity, shedding light on the immune properties which allow for the unique function of TSST-1 in mTSS.

Gen abstract type

Poster

Section Type

I&I

243 - Impact of growth media composition on antibiotic resistance profile of E. coli

Prerna Singh[1], Maria Boubia[1], Brandon Findlay[1]

[1]Concordia University

Antibacterial susceptibility testing is conducted according to established standardized guidelines to detect resistance and predict the clinical effectiveness of specific antibiotic treatments. Cation-adjusted Mueller-Hinton broth (MHB) is the most widely used bacteriological medium for minimum inhibitory concentration (MIC) determination due to its reproducibility and suitability for routine clinical testing. However, these standardized conditions do not fully replicate the complex and heterogeneous physiological environments encountered during infection, where factors such as nutrient availability, pH, osmolarity, host metabolites, and immune pressures can significantly influence antibiotic activity and bacterial survival. As a result, in vitro susceptibility measurements may not always accurately reflect in vivo treatment outcomes.

Urinary tract infections (UTIs) are among the most prevalent bacterial infections and represent a substantial burden on healthcare systems and antibiotic usage worldwide. In this study, we employed soft agar gradient evolution (SAGE) to generate nitrofurantoin-resistant *Escherichia coli* ATCC 25922 mutants (n = 16) in both artificial urine medium (AUM), which more closely mimics the urinary tract environment, and standard MHB. The choice of growth medium had a pronounced impact on evolutionary trajectories and resistance outcomes. While selection for nitrofurantoin resistance was observed under both conditions, the associated collateral responses to other clinically relevant antibiotics differed markedly between environments.

Specifically, mutants evolved in AUM exhibited cross-resistance to levofloxacin and trimethoprim, suggesting that the urinary tract-like environment may promote broader multidrug resistance profiles under selective pressure. In contrast, mutants evolved in MHB displayed collateral sensitivity to these antibiotics, indicating that standard laboratory conditions may underestimate or even reverse clinically relevant resistance interactions. Furthermore, AUM-evolved mutants demonstrated significantly reduced fitness costs, as evidenced by improved growth in the absence of nitrofurantoin compared with MHB-evolved mutants, suggesting that adaptation in physiologically relevant media may facilitate more stable resistance phenotypes.

Collectively, these findings suggest that commonly used laboratory media such as MHB may not fully recapitulate the physiological conditions present at infection sites, potentially limiting the predictive value of MIC-based susceptibility testing. This highlights the importance of incorporating infection-relevant media and environmental conditions when interpreting antibiotic susceptibility data and evaluating resistance evolution, in order to improve the clinical relevance of in vitro antimicrobial testing frameworks.

Gen abstract type

Poster

Section Type

I&I

244 - Strategic targeting of the *Salmonella* O-antigen to induce uptake by Kupffer cells using Phage22 or monoclonal antibodies

Lindsey Orthner[1], Wael Elhenawy[2], Brian Coombes[3], Stefanie Barbirz[4], Bart Bardoel[5], Bas Surewaard[1]

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Capture and eradication of bacteria that invade the bloodstream is a critical aspect of the innate immune system, performed by the strategically located liver macrophage population known as the Kupffer cells (KCs). The vast majority of Gram-positive and Gram-negative bacteria that enter circulation are efficiently taken up and cleared by KCs; however, bacteria that frequently cause infections have likely developed mechanisms to circumvent this process. We determined that pathogenic *Salmonella* spp. can evade this uptake. Often found in the human gut, this common pathogen has the ability to translocate from the gut to the bloodstream, enhanced by immune evasion and manipulation mechanisms, leading to invasive infections that cause lethal sepsis and recurrent infections in young children and immunocompromised individuals. Our data shows that, compared to other lipopolysaccharide (LPS)-containing Gram-negative bacteria, which are captured by KCs, the immune evasion mechanism delaying bacterial clearance and enabling the pathogen's persistence in the bloodstream relies on the unique *Salmonella* O-antigen (O-Ag). The O-Ag is part of the LPS on the bacterial cell surface and is suggested to be modulated by enzymes expressed by the lysogenic Phage22. It binds and uses this outermost region of the saccharide polymer as an entry receptor to infect *Salmonella*, subsequently altering the cell's surface. While P22 therapy offers a novel approach to combat antibiotic-resistant *Salmonella*, monoclonal antibodies (mAbs) targeting the O-Ag represents another promising alternative treatment that exploits this evasion mechanism and enhances pathogen clearance. In preliminary vaccination studies using attenuated strains, mice were protected from lethal challenge, and this protection was dependent on immunoglobulins. While vaccination with *ΔaroA* strains has been proposed in the past, there are clear limitations associated with the use of attenuated strains, particularly concerning biosafety. In contrast, mAb therapy is widely regarded as a safe and effective alternative, offering a more controlled and targeted approach with established safety profiles in clinical applications. We hypothesize that the *Salmonella* O-Ag, a key virulence factor that facilitates evasion of KC-mediated clearance, can be therapeutically targeted using phage therapy and/or mAbs. By specifically disrupting the protective functions of the O-Ag, these interventions would enhance KC recognition and clearance of *Salmonella* from the bloodstream, thereby reducing systemic dissemination. Biochemical and genetic techniques, including SDS PAGE silver staining, bacterial mutagenesis, and *in vivo* tracking of bacteria in murine liver sinusoids using intravital microscopy, were used to investigate this interaction. This research focuses on exploiting the *Salmonella* O-Ag using a new class of phage therapy and mAbs to modulate *Salmonella* clearance by KCs to fight the rising antibiotic resistance crisis.

Gen abstract type

Poster

Section Type

I&I

252 - Linking Microbial Community Structure to GABA Synthesis in Kefir

Emily Reitsma[1], Joseph Sebastian[1], Benjamin Willing[1]

[1]University of Alberta

Gamma-aminobutyric acid (GABA) is a bioactive metabolite derived from the amino acid glutamate and a primary inhibitory neurotransmitter in the central nervous system (Iorizzo et al., 2023). The metabolite is known for its antidepressant, neuroprotective, anti-inflammatory, antioxidant, and antihypertensive properties, as well as its ability to improve glycemic control and cholesterol levels, in addition to its calming and sleep-regulating benefits (Diez-Gutiérrez et al., 2020; Iorizzo et al., 2023). The primary site of GABA production is the cytoplasm of host neuronal and glial cells, catalyzed by the enzyme glutamate decarboxylase (GAD). However, GABA is also synthesized by gut-associated microbes, particularly lactic acid bacteria and certain yeasts, via glutamate decarboxylase (GAD) (Diez-Gutiérrez et al., 2020; Iorizzo et al., 2023). This GAD pathway, encoded by the *gadA* and *gadB* genes, converts glutamate to GABA while releasing CO_2 as a byproduct. An alternative pathway that utilizes and then converts the compound Putrescine to GABA also exists, though it is less prevalent and is primarily observed in yeast species (Diez-Gutiérrez et al., 2020).

In fermented food products such as kefir, microbial interactions play a role in metabolite production, including GABA production by kefir-associated yeast and bacteria. However, the competition dynamics among specific microbial communities in mixed-culture fermentation and their influence on GABA synthesis remain poorly understood, as competition for substrates and resulting interactions among microbes affect metabolic outputs.

Previous work from our lab has demonstrated that kefir produced from traditional grains or from a defined, pitched microbial consortium derived from the traditional kefir-grain community confers greater health benefits than commercially available kefir (Bourrie et al., 2021). Notably, both traditional and pitched kefir reduced plasma cholesterol (~35%) and liver triacylglycerols (~55%) in an obesity-induced mouse model, whereas kefir lacking either *Lactobacillus* spp. or yeast failed to produce these effects and performed similarly to a commercial product. Additionally, consumption of the pitched kefir product reduced LDL-C and inflammatory markers in hypercholesterolemic males over 4 weeks (Bourrie et al., 2023). These findings suggest that cooperative interactions between specific bacteria and yeast can generate bioactive metabolites, such as GABA, shown to be beneficial for host health.

Building on these findings, this study aimed to explore GABA production in these mixed culture kefir fermentations. Kefir was made using traditional grains, a pitched consortium, and modified consortia lacking either bacteria or yeast. Total RNA was extracted from each kefir and converted to cDNA for quantitative PCR (RT-qPCR) targeting glutamate decarboxylase genes (*gadA and gadB)*, which indicate GABA-producing potential of the inoculum. Additionally, GABA concentrations were directly quantified using chromatography techniques to assess functional metabolite output of each fermentation.

By linking microbial community composition to both *gad* gene expression and GABA production, this study aimed to provide insight into how microbial synergy within fermented food microbial communities influences the production of key bioactive compounds such as GABA. These findings may also help reinforce our understanding of the health benefits associated with traditionally structured kefir microbial communities and support the potential to optimize microbial community design for functionality in fermented foods.

Gen abstract type Poster

Section Type I&I

270 - Responding to a syphilis outbreak: Building capacity with automated RPR testing

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Background: Rising syphilis rates in Canada have required laboratories to perform much greater volumes of syphilis tests. Alberta's ongoing 2019 outbreak significantly increased demand for rapid plasma reagin (RPR) testing, straining a labour-intensive manual workflow and threatening timely reporting that is essential for prompt diagnosis and effective outbreak control. To address this, Alberta's Public Health Laboratory automated the RPR test. This study assessed the system-level impact of automation during a period of sustained outbreak-driven demand.

Methods: A six-year pre/post implementation study was performed from January 2019 to May 2025, spanning the 2023 introduction of automated RPR testing. Testing volumes and turnaround times were analyzed to assess performance under sustained demand growth. Manual and automated RPR workflows were compared, and workflow-based capacity modelling was used to estimate annual testing capacity for each approach.

Results: From 2019 to 2025, over 100,000 RPR tests were performed, with a 75% increase in annual demand. Capacity modelling predicted that automation could increase testing capacity by up to 221%, suggesting substantial headroom to absorb further demand. Consistent with these projections, implementation of automated RPR testing was associated with improved real-world laboratory performance, including: shorter average turnaround times; a higher proportion of results reported within 48 hours; and more consistent month-to-month reporting. Given the concurrent increase in test volumes, this indicated greater process stability overall.

Conclusions: In the setting of rapidly rising syphilis testing demand, RPR automation improved both reporting timeliness and operational consistency. Benefits extended beyond faster test results, reflecting a more stable and resilient testing system, even under sustained outbreak pressure. By increasing capacity and reducing sensitivity to surges in demand, RPR automation enabled more scalable and reliable syphilis testing and offers a practical model for laboratories facing similar workload pressures.

Gen abstract type

Poster

Section Type

I&I

272 - Early-life amoxicillin exposure drives *E. coli* phenotypic changes, translocation, and enhances host immune responses in later-life

Rajibur Rahman[1], Hannah Lantz[1], Janelle Fouhse[1], Stephanie Tollenaar[1], Erin Strachan[2], Tausha Prisnee[1], Michelle Park[1], Joshua Theriault[1], Cheng Long[1], Muhammad Aslam[1], Jonas Wong[2], Wael Elhenawy[2], Sue Tsai[2], John Harding[3], Benjamin Willing[1]

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Early-life antibiotic exposure can have lasting effects on immune function, but the microbial drivers and underlying mechanisms remain poorly defined. We previously showed that early-life antibiotic exposure is associated with a transient bloom of intestinal *Escherichia coli* in pigs and heightened inflammatory responses following intraperitoneal heat-killed *Salmonella* challenge later in life. Here, we investigated whether commensal *E. coli* contributes to long-term immune reprogramming and alters host defense against later-life live *Salmonella* infection.

In a neonatal piglet model, amoxicillin exposure during the first 14 days of life increased baseline systemic cytokines, including IL-8 and GM-CSF, at PND 35 prior to infection and enhanced inflammatory responses following *Salmonella* challenge, including increased IL-6 and IFN-γ. Antibiotic-exposed piglets exhibited reduced *Salmonella* shedding, lower incidence of diarrhea, and improved post-infection weight gain, indicating enhanced disease resilience. To determine microbial dependency, we used gnotobiotic mice colonized with an amoxicillin-resistant commensal *E. coli* strain (AR1) isolated from antibiotic-exposed piglets for subsequent functional analyses. These mice were exposed to antibiotics during early life via maternal treatment. Consistent with the pig model, antibiotic exposure during early life in the presence of this *E. coli* strain reduced *Salmonella* burden in the ileum, spleen, and liver following later-life *Salmonella* challenge, supporting an *E. coli*-dependent protective effect.

To define early changes underlying these effects, metatranscriptomic analysis of the intestinal *E. coli* community during antibiotic exposure revealed upregulation of fimbriae-associated and biofilm regulatory pathways. Consistent with these findings, this *E. coli* strain exhibited in vitro phenotypic remodeling characterized by increased motility, biofilm formation, and enhanced epithelial invasion (CMT-93 cells). We further observed that antibiotic exposure was associated with higher *E. coli* burden in extraintestinal tissues in both gnotobiotic piglets and monocolonized mice and was accompanied by elevated circulating IL-8 in piglets. In vitro, co-culture with Caco-2 cells further showed that antibiotic-exposed *E. coli* induced greater IL-8 secretion, supporting a conserved epithelial inflammatory response.

Consistent with these observations, amoxicillin + *E. coli* exposure reshaped immune cell composition in mice following *Salmonella* challenge. In the bone marrow, we observed expansion of innate dendritic cells alongside increased naïve $CD4^+$ T cells, with reduced CD38 expression and decreased CD38^hi dendritic cell subsets, consistent with a less activated myeloid state. In the spleen, exposed mice displayed altered T cell phenotypes (reduced naïve $CD4^+$ and increased $CD62L^-$ CD44^lo subsets) and increased MHCII^hi B cells, indicative of enhanced antigen-presenting capacity. Bone marrow-derived dendritic cells from mice prior to infection showed no differences in early uptake but exhibited reduced intracellular *Salmonella* burden at later time points, indicating enhanced intracellular bacterial clearance.

Collectively, these findings suggest that early-life antibiotic exposure promotes phenotypic remodeling of commensal *E. coli* and increases extraintestinal translocation, leading to immune reprogramming consistent with concepts of trained immunity and enhanced resistance to infection.

Gen abstract type Poster **Section Type** I&I

278 - Two cryo-EM structure-function studies: One pertaining to *Vibrio* effector protein interactions with F-actin and another regarding the motility of a bacterial predator

Mark Kreutzberger[1], Elena Kudryashov[2], Wanda Figueroa-Cuilan[3], Ravi Sonani[4], Dmitri Kudryashov[2], Mohammed Kaplan[3], Edward Egelman[4]

[1]University of Alberta, [2]The Ohio State University, [3]University of Chicago, [4]University of Virginia

The Kreutzberger lab is a new lab at the University of Alberta specializing in using cryo-electron microscopy (cryo-EM) in studies related to the structure and function of large biological macromolecular assemblies, particularly those related to bacteria and bacteria-host interactions. Here we share the results of the several recent studies which we think will be of interest to the bacterial community as well as advertise the new lab.

In the first study, we investigated the interactions of the *Vibrio parahaemolyticus* effector protein VopV with F-actin. VopV binds cytosolic F-actin (β/γ) isoforms with much higher affinity than it does to muscle (α) F-actin. Mutagenesis and cryo-EM structural investigation reveal isoform-specific residues with previously unidentified functions are responsible for these differences in binding affinity. These results have direct implication in *Vibrio parahaemolyticus* pathogenesis as its invasion of intestinal epithelial cells relies on the interaction of VopV with cytoplasmic F-actin.

In the second study, we investigated the swimming motility and flagellar structure of a bacterial predator, *Venatorbacter cucullus*, whose prey incudes pathogenic *Campylobacter jejuni* as well as *Escherichia coli*. Using fluorescence microscopy, we show that monotrichous *V. cucullus* has unique swimming motility punctuated by clear pauses in swimming and flagellar filament rotation rather than canonical flicking or wrapping of the flagella. These pauses mid run allow reorientations at small angular changes averaging ~29°. This behavior is imparted to *V. cucullus* by the extensive structural reinforcements in its mechanically rigid flagellar filament and flagellar hook assembled from “giant" subunits. Collectively, these results provide insights into how bacteria, including some predatory ones, can use large protein subunits to “fine tune” the properties of their flagella to alter their swimming behavior.

Gen abstract type

Poster

Section Type

I&I

292 - The effect of *Borrelia burgdorferi* infection on the microbiome of lab mice and ticks

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Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is maintained in nature through cycles involving vertebrate hosts and Ixodes ticks. In rodents, skin plays a central role in spirochete persistence and transmission to feeding ticks. Both host factors and vector-associated microbes influence these interactions. Recent studies suggest that tick feeding can temporarily disrupt the mammalian skin microbiome, and the tick gut itself harbors a diverse bacterial community crucial for tick physiology. Moreover, infection with *B. burgdorferi* or related *Borrelia species* can alter the composition of the tick microbiome, potentially shaping conditions that facilitate spirochete colonization.

To investigate the interaction between host infection, skin microbiota, and the tick microbiome, we analyzed four skin tissues (ear, dorsal, ventral, tail) and four nymphal ticks from 80 *Mus musculus* C57BL/6 mice that were either immunocompetent wild-type (WT) or severely immunodeficient (SCID) and experimentally infected through nymphal tick bite. Bacterial diversity was characterized using amplicon sequencing of the *16S rRNA* gene. In total, four nymphal tick sequencing libraries were processed, each library comprises of a tick that had fed as a larva on the corresponding mouse. These datasets enable integrated comparisons of both host-associated and tick-associated microbial communities within the same transmission system.

The study will test associations between *B. burgdorferi* infection status, host immune background, host sex, and the relative and absolute abundance of key bacterial taxa of interest, including members of the *Enterobacteriaceae*, *Rickettsia*, *Mycobacterium*, and *Rhodococcus*. The findings of this investigation will contribute to our understanding of the microbial interactions that may facilitate or hinder *B. burgdorferi* transmission.

Gen abstract type

Poster

Section Type

I&I

314 - Lipid adaptations in ceftriaxone-resistant *Neisseria gonorrhoeae* and its compensatory mutation

Julia Adug[1], Stephanie Bishop[2], Ian Lewis[1], Adriana Zardini Buzatto[1]

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Ceftriaxone-resistant *Neisseria gonorrhoeae,* the causative agent of the common sexually transmitted infection, gonorrhea, poses a concern as ceftriaxone is the last effective empirical treatment. According to the Gonococcal Antimicrobial Surveillance Program in Canada, there were 11 cases of ceftriaxone-resistant *N. gonorrhoeae* between 2023 and 2024, a significant increase from 5 cases reported between 2017 and 2021. Resistance typically arises from mosaic mutations in the penA gene, such as the *penA41* and *penA89* alleles, encoding penicillin-binding protein 2 (PBP2). While these mutations often incur fitness costs, compensatory mutations, such as an alanine deletion in a lysine transporter (formerly known as *mleN*ΔA467), can enhance the survival of resistant strains. However, the mechanism by which *mleN*ΔA467 restores fitness in biologically unfit ceftriaxone-resistant gonococcal strains is underexplored. This study investigates lipidome alterations in *N. gonorrhoeae* to elucidate lipid-based adaptive responses to resistance and the associated compensatory mutations.

Five isogenic strains of the FA19 *N. gonorrhoeae* strain harbouring either the *penA41*, *penA89*, or *mleN*ΔA467 and or a double mutant with *penA41 mleN*ΔA467 were grown in a chemically defined medium, Graver-Wade Medium, at 37˚C at 5% CO_2. Ceftriaxone resistance was confirmed using agar dilution. Culture aliquots were standardized to $1x10^9$ Colony Forming Units (CFU) and pelleted to collect intracellular lipids. A liquid-liquid extraction from a modified Folch method containing 0.2 M KCl, methanol and dichloromethane was performed. Lipidomic analysis was performed using Liquid-Chromatography-Mass Spectrometry.

Emerging evidence suggests a link between lipid remodelling and antimicrobial resistance (AMR), thereby altering bacterial membrane properties, which encompass phospholipids, cardiolipin, and other lipids. Mosaic *penA* allele mutations confer resistance, but reduce peptidoglycan transpeptidase activity, potentially affecting the integrity of the cell envelope. We hypothesize that mosaic *penA* alleles would result in membrane lipid remodelling due to compromised cell envelope integrity and would be reverted to wild-type levels upon introduction of *mleN*ΔA467, a fitness-compensatory mutation.

Lipidomic profiling revealed differences associated with the mosaic *penA* alleles, including effects consistent with bacteria undergoing the stringent response, which involves metabolic reprogramming, such as reduced phospholipid biosynthesis in response to stresses like resistance fitness costs. An observed accumulation of the phospholipid precursor, phosphatidic acid (PA), in the mosaic penA allele-only strains and a decrease in downstream phospholipids, such as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin (CL), reminiscent of the stringent response. This shows evident metabolic reprogramming in response to the fitness-cost stress associated with ceftriaxone resistance.

When the fitness-recovering *mleN*ΔA467 is introduced into the resistant *penA41* strain, we observed that the levels of PA, PE, PG, and CL were similar to those of the wild-type parent strain, displaying the opposite effect to that of the mosaic *penA* alleles alone. This shows that the compensatory mutation reverts this lipid-metabolism effect, enhancing the biological fitness of the ceftriaxone-resistant strains.

Our findings highlight that lipid remodelling is associated with recovery of biological fitness in ceftriaxone-resistant strains harbouring the *mleN*ΔA467 compensatory mutation. This study can serve as a foundation for future investigations into lipid-mediated fitness compensation mechanisms, enabling the design of lipid-targeted diagnostics and therapies to combat AMR.

Gen abstract type Poster **Section Type** I&I

316 - High throughput functional genomics: microdroplet profiling of ulcerative colitis microbiome for *Enterobacteriaceae pathobionts*

Isaac Framst[1], Rana Özcan[2], Lindsay Kalan[1], Tohid Didar[2], Michael Surette[1]

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Inflammatory bowel disease (IBD) is a lifelong disease with high morbidity and economic burden, and will affect nearly half a million Canadians by the end of the decade. Enterobacteriaceae in the intestinal mucosa of IBD patients contribute to disease, a process driven by strain-specific virulence factors that facilitate colonization and disrupt the intestinal epithelium. A detailed understanding of these factors is critical for antibiotic and fecal microbiota transplantation (FMT) treatments in IBD. We have previously demonstrated that culture-enrichment can detect these strain-specific markers, but is impractical for large cohort studies and patient screening. Microdroplet-based culture enables compartmentalization of single bacteria, eliminates competition, and is high-throughput. We propose that microdroplet culture could replace traditional methods, offering greater efficiency at a fraction of the cost. Applications in direct functional screening of complex samples and isolation of single bacteria, even low- abundance ones, are also far more practical with microdroplets. We developed a microdroplet-based method for massively parallel, highly sensitive targeted culture-enriched metagenomics of Enterobacteriaceae and functional screening of biomarkers to identify Enterobacteriaceae pathobionts in ulcerative colitis patients. We demonstrate the capabilities of this droplet workflow for selective enrichment of *Enterobacteriaceae* from stool and for culture enrichment in droplets, which recovered greater diversity while dramatically reducing workload under anaerobic conditions. We also conducted functional screening of mixed microbial samples for specific β-1,4-N-acetylglucosaminidase and sialidase activity at the single-cell level in droplets. These served as a proof-of-principle for high-throughput culture and screening applications in droplets. Future work will develop fluorescence-activated droplet sorting to isolate droplets containing functions and genes of interest to further characterize pathobionts and isolate beneficial bacteria associated with remission in IBD. We will then apply droplet culture and screening methods to an FMT cohort study to detect microbial features associated with remission in UC patients. This work demonstrates the power of droplet-based methods for high-throughput and low-cost bacterial culture and functional screening, and can be applied to a wide variety of applications in human, animal and environmental microbiome studies.

Gen abstract type

Poster

Section Type

I&I

317 - The impact of a defined microbial community on *Enterococcus cecorum* infection in broiler chickens

Dajana Lleshi[1], Usha Sivakumar Sharma[1], Camila Schultz Marcolla[1], Rajibur Rahman[1], Stephanie Tollenaar[1], Tomohiro Hamaoka[2], Benjamin P. Willing[1]

[1]AFNS, University of Alberta, Edmonton, AB, Canada, [2]Asahi Biosciences, Georgia, USA

Infectious skeletal disease caused by *Enterococcus cecorum* has become a major concern in the broiler industry. *E. cecorum* is a gram-positive, facultative anaerobic bacterium that is normally part of the chicken gut microbiota, however pathogenic strains can translocate beyond the intestine and are associated with disease. Infection is characterized by vertebral lesions, lameness, paralysis, sepsis and increased mortality. This highlights the need for alternatives to antibiotic treatments while supporting animal health and improving production efficiency. In this study, we evaluated the effect of a defined microbial community (MC) on pathogen load following an oral infection. The MC consisted of 30 cultured bacterial isolates originally isolated from the adult chicken cecum, representing the phyla Bacillota, Bacteroidota, Pseudomonadota, and Actinomycetota. Day-old chicks (n = 36) were orally inoculated with the MC and challenged the following day with a pathogenic field isolate of *E. cecorum*. Bacterial loads were quantified by culturing liver tissue and femoral head swabs at days 7, 14, and 21. MC treatment reduced *E. cecorum* counts in the liver, with significant reductions at day 14 ($p = 0.027$) and day 21 ($p = 0.023$), indicating decreased systemic spread. In the femoral head, bacterial load was significantly lower at day 7 ($p = 0.018$), with a continued downward trend by day 21, suggesting early limitation of colonization at the primary site of infection. Overall, these findings suggest that the defined microbial community can reduce *E. cecorum* colonization and may contribute to improved resilience during infection.

Gen abstract type

Poster

Section Type

I&I

318 - Hidden Reservoirs of Antibiotic Resistant *Clostridioides difficile*: A Metagenomic Study of Asymptomatic Human and Animal Gut Microbiomes

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Introduction: *Clostridioides difficile* is a leading cause of healthcare-associated diarrhea globally. *C. difficile* infection (CDI) is commonly treated with metronidazole, vancomycin, fidaxomicin, and occasionally rifaximin. Reduced susceptibility to these antibiotics has increasingly been reported, primarily among clinical isolates from symptomatic CDI patients. Antibiotic resistance in *C. difficile* is largely driven by single-nucleotide polymorphisms (SNPs) in key genes, including the *nimB* promoter (metronidazole), *vanR*/*vanS* (vancomycin), *rpoB* (rifaximin), and *rpoC* (fidaxomicin). Epidemic strains commonly also harbor *gyrA* mutations conferring fluoroquinolone resistance. However, the prevalence of resistant *C. difficile* in asymptomatic hosts without prior CDI treatment remains poorly characterized. This study aims to assess the occurrence of antibiotic-resistant *C. difficile* within gut metagenomes of asymptomatic or non-CDI human and animal hosts.

Methods: SRA datasets containing possible *C. difficile* were screened and only human and animal gut SRA datasets were retrieved and assembled using Logan. The presence of six resistance-associated genes (*PnimB*, *gyrA*, *vanR*, *vanS*, *rpoB* and *rpoC*) was detected using TBlastN with gene-specific thresholds, and resulting matches were aligned using MAFFT. SRAs with resistance-conferring SNPs were confirmed as *C. difficile* by BLAST and their metadata extracted.

Results: A total of 1,048,576 SRA datasets were screened, of which 16,220 were identified as containing *C. difficile*, including 14,306 human gut and 1,914 animal gut datasets. Among human samples, 3,065 datasets matched at least one of six resistance associated genes. Of these, 14 (0.46%) carried the metronidazole associated P*nimB* SNP, 6 (0.20%) carried the vancomycin associated *vanR/vanS* SNP, 22 (0.72%) carried the rifaximin associated *rpoB* SNP, and 89 (2.90%) carried the fluoroquinolone associated *gyr*A SNP. Similarly, among animal datasets, 423 samples matched at least one resistance gene; 4 (0.95%) carried the P*nimB* SNP, and 9 (2.13%) carried the *gyrA* SNP. No dataset contained resistance-associated mutations for fidaxomicin. These findings indicate that asymptomatic or non-CDI humans and animals can harbor *C. difficile* strains that are already resistant to antibiotics commonly used to treat CDI. Such hosts may act as reservoirs, increasing the risk of transmission of resistant strains to patients. This may contribute to CDI cases in which resistance is present prior to treatment, potentially leading to poorer clinical outcomes.

Gen abstract type

Poster

Section Type

I&I

332 - Characterizing the intracellular fate of modern *Acinetobacter baumannii* isolates in host cells

Enrique Aburto Arreguin[1], Ninad Mehta[2, 3], Jessica R. Sheldon[1]

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The opportunistic pathogen *Acinetobacter baumannii* has accrued a reputation worldwide for causing recalcitrant nosocomial infections due to its ability to rapidly acquire antibiotic resistance factors, including against last-resort antibiotics such as carbapenems and colistin. While a great deal of research has focused on its antibiotic resistance mechanisms and global prevalence of epidemic clones, research pertaining to how *A. baumannii* interacts with the host during infection is comparatively scarce. To date, the majority of *A. baumannii* pathogenesis studies have been conducted using two reference strains, ATCC 17978 and ATCC 19606, which were both isolated more than 70 years ago. Many in the *A. baumannii* field have begun to question whether these two reference strains present the most faithful models of virulence seen with modern isolates of *A. baumannii*, and as such, have pushed for greater usage of these strains in pathogenesis studies. Based on emerging literature, we hypothesized that, when compared to laboratory-adapted reference strains, modern *A. baumannii* clinical isolates have enhanced virulence potential against host cells, mainly in their ability to persist and/or replicate within various cell types. The reference strain 17978, and four modern isolates LAC-4, PHO-1, RUH-2, and RUH-21, were selected and screened against RAW264.7 murine macrophages using gentamicin protection assays, with apramycin being used as an alternative antibiotic when screening gentamicin-resistant isolates. Three of the four modern isolates examined were shown to maintain intracellular colony-forming units per milliliter (CFU/mL) at 8 and 24 hours post-infection, with less than a 1 log reduction relative to their initial uptake counts, whereas RUH-21 and the type strain 17978 suffered 1.5 and nearly 3-log reductions in intracellular CFU/mL over the same timepoints, respectively. The panel of clinical isolates is currently being expanded to include strains AB5075, HUMC1, and RUH-14, to gain a more comprehensive understanding of the intracellular fate of *A. baumannii*. Stable fluorescent labelling of all *A. baumannii* strains is being used to assess their intracellular localization and replicative capacity in fluorescence-based proliferation assays. Several other avenues will be explored in future work, including extending the length of screens to determine the time to total eradication of intracellular bacteria, if at all. Screens will also be expanded to another macrophage cell line (THP-1) in order to determine if these results can be replicated in a more human-relevant cell culture model and are not a cell line artifact unique to RAW264.7 cells. Additionally, the A549 and HMEC-1 cell lines will be utilized to assess how our chosen panel of *A. baumannii* isolates interacts with epithelial and endothelial cells, respectively. These models will further be used to determine whether intracellular persistence in these isolates provides a protective niche that shields bacteria from extracellular stressors, including antibiotics and innate immune effectors. Ultimately, this work seeks to advance our understanding of how host cells, as potential intracellular reservoirs for *A. baumannii,* may contribute to the persistence of antibiotic-resistant infections commonly seen with this bacterium.

Gen abstract type

Poster

Section Type

I&I

333 - Translational Development of Synthetic α-Helical Antimicrobial Peptide-Bacteriophage Combination Therapy Against Biofilm-Associated Prosthetic Joint Infection (PJI) Pathogens

Md Ramim Tanver Rahman[1, 2], D. William Cameron[1, 3], Robert Feibel[4], Melissa T Cammuso[5], John C. Bell[1], Leanne Mortimer[6], Yang Yu[6], Bradley W. M. Cook[7], Marisa Ann Azad[1, 3]

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Background: Periprosthetic joint infection (PJI) is a severe complication of orthopedic surgery, frequently associated with biofilm-forming multidrug-resistant pathogens that colonize prosthetic surfaces. These sessile microbial communities are embedded within an extracellular polymeric substance (EPS) matrix and often exhibit reduced metabolic activity, thereby limiting susceptibility to host immune clearance and systemic antimicrobial therapy. Clinical management is further complicated in patients with immunocompromise or antibiotic hypersensitivity, underscoring the need for alternative antimicrobial strategies. In this context, the present study evaluates the translational potential of a combinatorial therapeutic strategy integrating rationally designed synthetic α-helical antimicrobial peptides (AMPs) with targeted lytic bacteriophages. By coupling the rapid, broad-spectrum membrane-disruptive activity of AMPs with the strain-specific biofilm penetration, extracellular polymeric substance (EPS) degradation, and bactericidal activity of phages, this multimodal approach is designed to overcome established resistance mechanisms and eradicate mature biofilms associated with clinical PJI. **Objectives:** To characterize the synergistic antimicrobial and antibiofilm activity of AMP-phage combination therapy and evaluate its hemocompatibility for translational application in orthopedic infection management. **Methods:** We rationally designed a series of α-helical amphipathic peptides (M-5W0A, M-3W2A, M-2W1A, and M-3W2A-i) optimized for membrane disruption and proteolytic stability via N-terminal capping and C-terminal amidation. Design was supported by molecular docking against bacterial targets (e.g., MurB) and structural confirmation via circular dichroism (CD). Antibacterial efficacy was established by determining MICs against clinical PJI isolates, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE) and *S. epidermidis*. Standard and clinical isolates were challenged with lead AMP in combination with biobank-derived lytic bacteriophages. Synergy and killing kinetics were quantified via checkerboard microdilution and time-kill assays. Biofilm eradication on stainless steel Kirschner wires was assessed using crystal violet, resazurin, and CFU enumeration. Translational safety was defined by human erythrocyte hemolysis assays to establish the therapeutic window. **Results:** The engineered AMPs, specifically lead candidate M-5W0A, exhibited potent broad-spectrum activity with MICs ranging from 2-512 µg/mL. Exceptional efficacy was observed against *Corynebacterium striatum* (2 µg/mL) and *S. epidermidis* (8 µg/mL), with maintained potency against highly resistant MDR strains, including vancomycin-resistant *Enterococcus faecium* (32 µg/mL) and MRSA (128 µg/mL). Computational docking analysis corroborated these findings, indicating high binding affinity for critical bacterial targets such as MurB. In combination with lytic phages, synergistic regimens achieved enhanced lethality compared to monotherapy, characterized by accelerated suppression of both planktonic and sessile populations at reduced effective concentrations. On implant-relevant 316L stainless steel surfaces, the combination therapy demonstrated significant capacity to penetrate and eradicate mature biofilms, achieving up to an 85% reduction in metabolic activity and multi-log reductions in viable bacterial load. Importantly, preliminary biocompatibility assays confirmed a favorable safety profile, with minimal erythrocyte toxicity at concentrations exceeding bactericidal levels, establishing a viable therapeutic window for clinical translation. **Conclusion:** This study establishes a preclinical framework for AMP-bacteriophage combination therapy as a next-generation strategy for hardware-associated PJI. By coupling the rapid membrane-disruption of synthetic peptides with the precision targeting of our clinical phage biobank, this platform addresses current antibiotic limitations while preserving host safety, supporting progression toward human therapeutic application.

Gen abstract type Poster

Section Type I&I

341 - Evaluating Nanopore Long-Read Sequencing of Enrichment Cultures to Reveal the Hidden Resistome in Canadian Seafood

Derek D.N. Smith[1], Kloé Neszvecsko[2], Audrey Charlebois[2], Lisa A. Johnson[3], David Huyben[4], F. Carl Uhland[2], Carolee A. Carson[2], Richard J. Reid-Smith[2], Dounia Hamoutene[3]

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The burden and trends of antimicrobial resistance (AMR) within farmed and wild caught Canadian seafood remain poorly understood. Prior efforts through the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) have been focused on retail packaged foods as significant logistical constraints have limited sample collection and data on AMR and antimicrobial resistance genes (ARGs) directly linked to source environments. These efforts have also focused on human-health priority zoonotic or commensal bacterial species; consequently, the carriage of mobilizable ARGs in the broader host microbiota, including emerging pathogens, is an underexplored area that represents a significant gap under a One Health surveillance approach.

As part of the Genomics Research and Development Initiative's Shared Priority Project on One Health AMR, we sought to bridge this gap with three specific aims: 1) to profile AMR from samples sourced directly from aquatic environments; 2) to identify mobilizable ARGs within non-target host microbiota; and 3) to evaluate the utility of long-read enrichment metagenomics as a One Health surveillance tool. To achieve this, we conducted a pilot study profiling isolates and enrichment cultures from diverse Canadian aquatic hosts: farmed rainbow trout (*Oncorhynchus mykiss*, Lake Huron), farmed Atlantic salmon (*Salmo salar*, Bay of Fundy) and wild Atlantic lobster (*Homarus americanus*) caught near farms and in reference areas, as well as wild and farmed bivalves (oysters - *Crassostrea virginica*, mussels - *Mytilus edulis,* Prince Edward Island). Tissue, feces, gill and mucosa samples were processed using CIPARS enrichment and isolation methodologies for seafood samples. Sufficient growth was achieved for 176 samples in Tryptic Soy Broth (TSB) and 184 in Alkaline Peptone Water (APW). Targeted isolation recovered *Escherichia coli* (n=13), *Aeromonas spp*. (n=32), and *Vibrio spp*. (n=88). Antimicrobial susceptibility phenotypic profiling with Sensititre Gram-negative NARMS plates identified second- and third-generation cephalosporin and macrolide resistance in two *Aeromonas* isolates from rainbow trout.

To characterize the broader genomic resistome, DNA from isolates and enrichments is being sequenced on the Oxford Nanopore P2 Solo platform (R10.4.1 flow cells, Kit 14 Rapid Barcoding, super accuracy basecalling). Isolates are being assembled via Hybracter, while nf-core/MAG is being used to recover metagenomic assembled genomes (MAGs) and extrachromosomal elements from the enrichments. Downstream analysis will identify ARGs in both isolates and MAGs from non-target microbiome species, compare core genomes across MAGs and isolates for phylogenetics, and assess whether isolates represent target species diversity within enrichments. The results from this pilot study will evaluate the utility of enrichment culture metagenomics to inform future efforts in One Health surveillance for AMR and pathogens.

Gen abstract type Poster

Section Type I&I

346 - A DNA binding protease regulates lysogeny and prophage immunity in Arthrobacteriophages

Katia Koziel Ly[1], Zach Mitchell[1, 2], Leen Madani[1], Elizabeth Williams[1], Adam Rudner[1]

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For decades, the long-standing mechanism for lysogen maintenance was found in phage Lambda and relies on the cI, immunity repressor (IR) protein. The IR recognizes phage DNA and suppresses the lytic cycle. Additionally, the phages require an integrase protein that integrates its DNA into the bacterial chromosome. Therefore, when a phage encodes an immunity repressor and an integrase, the phage is hypothesized to follow IR-mediated lysogeny.

uOttawa PhageHunters have isolated and sequenced nineteen AZ cluster bacteriophages. These phages were experimentally shown to form lysogens but do not encode a homolog to the IR protein. Superinfection immunity (SI) was observed against phages on the Pixelle and Amyev lysogens and several phages were observed to rapidly acquire mutations that allowed them to escape SI, which we deemed defense escape mutants (DEMs). These DEMs of ObiToo and Crewmate shared a mutation upstream of a conserved DNA binding protein, SigAZ, that has homology to a RpoS stress-activated sigma factor from *E. coli*. We propose that SigAZ is the first member of an immunity cassette in AZ phages that ends with a conserved serine integrase, and contains a conserved DNA-activated protease, ThbSP, that has homology to the eukaryotic Spartan protease.

We will investigate a model where ThbSP binds to the promoter of SigAZ and proteolytically cleaves it into an inactive form to promote lysogeny. We have created inducible expression plasmids for *Arthrobacter globiformis* and have shown that in the presence of SigAZ and ThbSP, infection increases and decreases, respectively. Our future work aims to (1) delete ThbSP from an AZ phage, (2) examine the ThbSP binding site, and (3) observe ThbSP protease activity against SigAZ.

Our work will unveil a novel mechanism of lysogeny that will expand our understanding of phage life cycles and encourage empirical testing of phages for lysogeny.

Gen abstract type

Poster

Section Type

I&I

351 - Investigating *Salmonella enterica* SspH2's interactions with host adaptor protein Tollip

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Introduction: *Salmonella enterica (S. enterica)* is a prominent gastrointestinal pathogen, with clinical presentations ranging from self-limiting diarrhoeal disease to life-threatening systemic infection. Currently estimated to cause over 250,000 deaths per year, *S. enterica's* burden on public health systems is exacerbated by the rising prevalence of antimicrobial resistance, with the World Health Organization classifying *S. enterica* as a high-priority pathogen in 2024. To meet the need for novel therapeutics to combat *S. enterica* infection, it is imperative to develop a robust understanding of the pathogenic strategies *S. enterica* employs and identify virulence factors that could pose as potential targets for anti-virulence treatments.

As a facultative intracellular pathogen, *S. enterica* relies on effector proteins that allow the bacteria to establish a niche inside host cells. These effectors are secreted directly into the host cytoplasm via a type III secretion system, though the intracellular actions of many of these effectors are not fully understood. One effector of interest, SspH2, is a highly conserved E3 ubiquitin ligase that associates with the plasma membrane of the host cell and subverts the host's ubiquitin regulatory system to directly ubiquitinate host proteins, potentially impacting that target protein's activity.

Objective: In order to understand SspH2's role in *S. enterica* pathogenesis, we aimed to identify host substrates ubiquitinated by SspH2, and characterize the consequences for host cells.

Results: To probe SspH2-host interactions, our group has developed a monocytic cell line that inducibly expresses SspH2 and a tandem cell line expressing a catalytic mutant SspH2^{C580A}, which abrogates ubiquitination activity. We performed an initial mass spectrometry screen for differentially ubiquitinated proteins between the two cell lines to identify possible substrates of SspH2. Lysine 150 (K150) of Toll-interacting protein (Tollip) was ubiquitinated significantly more in cells expressing SspH2 compared to those expressing SspH2^{C580A}. Using co-immunoprecipitation, we were able to validate that Tollip and SspH2 interact. Tollip K150 has been implicated in autophagosome-lysosome fusion and anti-inflammatory functions through its ability to bind lipids. To explore the implications of SspH2-mediated ubiquitination of Tollip we sought to interrogate its impact on these two Tollip functions. Upon examining the inflammatory profile of our SspH2-expressing cells, we observed an increase in production of the proinflammatory cytokine IL-1β. In assessing SspH2's impact on autophagy, we observed an accumulation of LC3-I indicative of autophagy impairment. Interestingly, LC3-II did not accumulate, suggesting that SspH2 may be hindering autophagosome formation, rather than blocking their fusion with lysosomes.

Conclusion: By characterizing SspH2-Tollip interactions and their physiological consequences, we can better understand how SspH2 contributes to *S. enterica* pathogenesis and garner more insights into the intracellular lifestyle of the bacteria. Identifying these host-pathogen interactions and understanding their implications in disease acts as a foundational step in the development of novel therapies designed to interfere with these interactions and ultimately alleviate the burden *S. enterica* imposes on public health.

Gen abstract type Poster

Section Type I&I

352 - PI(3,4)P2 involved in macrophages surveillance mechanisms

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Macrophages (MΦs) sense and respond to pathogens and microbial molecular associated patterns, including threats our bodies encounter daily. These functions rely on highly specialized plasma membrane (PM) biochemical signaling and actin dynamics largely controlled by phosphoinositide species (PIs). PIs are signaling lipids on the cytosolic leaflet of the PM or intracellular compartments that, depending on their phosphorylation state, carry out specific cellular functions, including the regulation of cell membrane and actin dynamics, and the intracellular trafficking, among others.

Macrophage response toward soluble stimuli has been extensively investigated at a time of hours after stimulation. But knowledge about any immediate response at a second-to-minute timescale has been absent. Here, we investigated the role of plasma membrane PIs role in sensing soluble microbial cues.

We herein report that upon contact with Gram-negative filter-sterilized media, macrophages RAW 264.7 cells readily respond producing a peak of the phosphoinositide PI(3,4)P2 at the plasma membrane that correlates with changes in actin dynamics and micropinocytosis, which depends on the lipid kinase class I PI3kinase.

Together, these results suggest a model in which the transient PI(3,4)P2 peak functions downstream a MΦs rapid sensing mechanism for microbial presence prompting the cells to maximize surveillance capacity for invasive microbes. Defining the molecular triggers and signaling components involved may reveal a previously unknown layer of innate immune surveillance based on plasma membrane lipid signaling and cytoskeletal remodeling.

Gen abstract type

Poster

Section Type

I&I

353 - Skin-Sourced Staphylococcal Phages for Treatment of Periprosthetic Joint Infections

Emily Wood[1], Celina Tanbari[1], Kieran Furlong[1], Elizabeth Williams[1], Adam Rudner[1]

[1]University of Ottawa

Medical device-associated infections are among the most common hospital-acquired infections, representing a major challenge in modern healthcare. Periprosthetic joint infections (PJIs), occurring after joint replacements, are a common and particularly destructive infection, with more than 50% caused by *Staphylococcus* species.

Staphylococcus epidermidis, a frequent culprit, is an opportunistic pathogen normally found on the skin, that becomes infectious when it colonizes implanted medical devices within the body. Unlike other *Staphylococcus* species, biofilm formation is the primary virulence factor of *S. epidermidis* in infections. Biofilms are complex structural communities that evade of host-immunity and antibiotics. Rising antimicrobial resistance (AMR) and the resistant nature of bacterial biofilms highlights an urgent need for alternative treatments, with phage therapy (PT) emerging as a promising solution.

Bacteriophages (phages) are viruses that infect bacteria, and are incredibly abundant in nearly every environmental niche, including the human skin microbiome. However, phages are highly specific, and therapeutic use relies on access to large, diverse libraries to find effective matches against clinical isolates. Among 206 phages reported in literature to infect coagulase-negative *Staphylococci*, only 82 target *S. epidermidis*.

This study aims to expand the availability of *S. epidermidis* bacteriophages by generating a biorepository with potential for PT use.

Since May 2024, in conjunction with an undergraduate bacteriophage discovery lab, we have characterized 79 novel phages from human skin swab samples against 8 *S. epidermidis* strains. We are sequencing these phages with Nanopore long-read sequencing with preliminary results demonstrating genomic diversity within our collection among 3 clusters. To identify PT candidates, we have evaluated host range, virulence, and anti-biofilm activity among a subset of ten lytic phages. Further work will test promising candidates in conjunction with antibiotics and purified endolysins, and against clinical isolates from patients with persistent PJIs at The Ottawa Hospital. This will provide insights into the in vivo efficacy of these phages, and their potential use in the clinic.

We are employing a direct-from-plaque sequencing method that allows us to invert the standard phage hunting workflow in order to increase the diversity of our phage collection. At the CSM2025 conference we solicited samples from attendees, returned to Ottawa and isolated *S. epidermidis* bacteriophages on several hosts, sequenced 95 plaques and archived six unique phages into our collection.

Gen abstract type Poster

Section Type I&I

357 - Systematic identification of novel molecular modulators of host-ubiquitin signalling by *Legionella pneumophila*

Cathy Le[1], Shun-Je Bhark[2], Deepak T Patel[1], Nobuhiko Watanabe[1], Jonathan Pruneda[2], Alexei Savchenko[1]

[1]Department of Microbiology, Immunology and Infectious Diseases University of Calgary, Calgary AB, Canada, [2]Department of Molecular Microbiology & Immunology, Oregon Health & Science University, Portland, OR, USA

Ubiquitination is a highly conserved and eukaryote-specific post-translational modification. Classical ubiquitination is a three-step cascade catalyzed by three enzymes: the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and an E3 ligase. In the human genome, over 600 E3 ligases have been identified, making them the largest and most functionally critical components of the ubiquitination process, as they not only select ubiquitination targets but also influence the type of lysine-linked polyubiquitin chains. Given this level of control, it is not surprising that the dysregulation of E3 ligases has been implicated in a wide range of human diseases, including cancer, Alzheimer's, and Parkinson's. These links to diseases have led to increased research efforts to better understand the catalytic mechanisms of E3 ligases as well as to harness them for therapeutic innovation and applications.

However, humans are not the only ones, nor the first ones, who have recognized the power of E3 ligases. Despite lacking their own conventional ubiquitination system, pathogenic bacteria have evolved specialized secretion systems that enable them to translocate virulence proteins, known as effectors, to manipulate or subvert key host cell signalling cascades to ultimately promote their survival and replication. One such bacterium is *Legionella pneumophila*, a Gram-negative intracellular pathogen that causes Legionnaires' disease, a fatal form of pneumonia. Human exposure to this bacterium occurs through the inhalation of *Legionella*-contaminated water aerosols, such as those from showers and air-conditioning systems. Within the lung, *L. pneumophila* infects alveolar macrophages and prevents their maturation into the degradative phagolysosome, instead converting the phagolysosome into a growth and replication organelle known as the *Legionella*-containing vacuole. To accomplish this, *Legionella pneumophila* uses its type IV secretion system to deliver over 360 effector proteins into alveolar macrophages, thereby manipulating and subverting key host-cell signalling cascades to support its intracellular growth and replication. This is the largest known effector arsenal in the bacterial world; however, many of these effectors remain uncharacterized due to challenges with forward genetic approaches and a lack of sequence similarity to functionally annotated proteins. Additionally, in our previous work, where we annotated the predicted functional domains of the *L. pneumophila* translocated effectors, we were unable to identify any new E3 ligases based on the predicted protein model of the *L. pneumophila* effectors.

To address this gap and discover *L. pneumophila* effectors that target the host ubiquitination machinery, we implemented a high-throughput functional screen that detects E3 ligase activity independent of sequence or structural homology. Screening 155 *L. pneumophila* effectors, we identified 2 previously uncharacterized, structurally novel E3 ligases. The identification of these newly annotated effectors not only provides insights into *Legionella pneumophila*'s manipulation of the host but also acts as a guide to systematically discover novel ubiquitination machinery E3 ligases in other bacterial pathogens. Understanding how bacterial pathogens hijack the human ubiquitination pathways provides a foundation for synthetic biology approaches that can be leveraged to not only disrupt bacterial infection but also to reprogram the bacterial E3 ligases into novel therapeutic tools for human disease.

Gen abstract type Poster

Section Type I&I

358 - Disarming *Legionella pneumophila* Effectors with de novo designed metaeffectors

Deepak Patel[1], Cathy Le[1], Lukasz Jaroszewski[2], Adam Godzik[2], Alexei Savchenko[1]

[1]University of Calgary, [2]University of California, Riverside

Legionella pneumophila is a Gram-negative bacterium that parasitizes phylogenetically diverse freshwater unicellular amoebae. Therefore, freshwater environments have served as an 'evolutionary playground' for this bacterium to evolve an extensive molecular toolkit to manipulate eukaryotic cellular processes - many of which are present in human alveolar macrophages. Consequently, human inhalation of contaminated aerosols generated from a poorly maintained freshwater system allows *L. pneumophila* to enter the lower respiratory tract. Within this environment, *L. pneumophila* replicates and grows inside alveolar macrophages, which can then manifest into a life-threatening form of pneumonia known as Legionnaires' disease. A critical tool for *L. pneumophila* is the Type IVB Dot/Icm (defective in organelle trafficking/intracellular multiplication) secretion system - a 27-component molecular nanomachine that acts as a syringe to facilitate the delivery of over 360 pathogenic factors, called "effectors", into the host cytosol. Specifically, effectors orchestrate all aspects of the pathogen's lifecycle by modulating a myriad of host cellular processes, such as protein translation, autophagy, transcription, vesicle trafficking, cytoskeleton dynamics, and cellular respiration. An important aspect of this pathogenic strategy involves the regulation of effector activity by proteins known as metaeffectors ("effectors of effectors"). To date, more than ten effector-metaeffector pairs have been identified, revealing three primary mechanisms of regulation: 1) reversal of effector-mediated host protein modifications; 2) direct modification of the effector to inhibit its function; and 3) inhibition of effector function through direct protein-protein interactions. By using the third mechanism as a molecular blueprint, I applied de novo protein design tools, such as BindCraft, to generate minibinder proteins that function as synthetic metaeffectors. Here, I present a proof-of-concept in which structure-guided designs mimic the inhibitory function of the Lpg0945/LegL1 metaeffector against the Lpg0944/RavJ effector. Overall, this work establishes the foundational basis of designing novel protein-based therapeutics against *L. pneumophila,* with broader application to multi-drug resistant bacterial pathogens, including *Salmonella* and *Shigella* species.

Gen abstract type

Poster

Section Type

I&I

359 - Investigating Potential Mechanisms of Resistance to Carvacrol in *E. coli*

Cameron Deleeuw[1], Delaney Huhtala[1], Sahiba Pahwa[1], Randi Guest[1], Benjamin Bourrie[2]

[1]MacEwan University, [2]MacEwan University and University of Alberta

The antibiotic crisis is a growing concern, with a lack of alternatives used to treat infection. Carvacrol, an essential oil found in oregano has been found to have antimicrobial properties, largely through the disruption of microbial membranes. While there are a number of studies examining the antimicrobial activity of carvacrol, the potential development of resistance has not been thoroughly examined. Particularly, our understanding of potential bacterial mechanisms of resistance to this compound are still poorly understood. This project sought to investigate the potential for the development of microbial resistance to carvacrol using *Escherichia coli* as a model organism. We first established a minimum inhibitory concentration of carvacrol for *E. coli,* then conducted a mutagenesis assay, generating 2 mutants with significantly increased carvacrol resistance when compared to the parent strain. These mutants, along with the parent strain, then underwent high molecular weight DNA extraction followed by whole genome sequencing using Oxford Nanopore Technology long read sequencing at ~150x coverage. Reads were quality filtered and mapped to a reference genome of *E. coli* (BW25113 - CP009273) for all three strains. Variants were called for each strain and mutations with >70% frequency were compared between resistant mutants. This analysis revealed the presence of 3 shared mutation between the two resistant mutants, (marR, thrS, and yadC). MarR is a repressor of the multiple antibiotic resistance operon, indicating that the likely mechanism of resistance is related to expression of multi-drug efflux pumps. These results indicate that resistance to carvacrol can be developed by organisms, even if it is not common. Additionally, the results point to the potential for resistant microbes to develop cross-resistance to other antimicrobial compounds along with carvacrol, representing a significant concern regarding carvacrols use as an alternative antibiotic. Future work will include confirming the resistance phenotype associated with marR mutation in a sensitive background and investigating the potential for this mutation to result in multi-drug resistant strains.

Gen abstract type

Poster

Section Type

I&I

363 - A Comparative Study of Honey and Propolis in Enhancing Bacteriophage Therapy for MDR Biofilm Eradication in *Escherichia coli* and Klebsiella pneumoniae

Maria Alejandra Rojas Rivero[1], Naowarat Cheeptham[1], Kingsley Donkor[1], Karen Fong[2], Heidi Huttunen-Hennelly[1]

[1]Thompson Rivers University, [2]Summerland Research and Development Centre

Biofilm-associated infections caused by multidrug-resistant (MDR) bacteria pose a significant challenge in clinical settings due to their enhanced tolerance to antimicrobial agents and host immune defenses. These infections are particularly difficult to treat, as bacteria embedded within biofilms exhibit altered metabolic states and are protected by an extracellular polymeric matrix that limits antimicrobial penetration. As a result, alternative or adjunct therapeutic strategies are urgently needed. This study investigated the antimicrobial and antibiofilm efficacy of natural products: Manuka honey, raw honey, and propolis, both individually and in combination with bacteriophages and the antibiotic ciprofloxacin against MDR *Escherichia coli* and *Klebsiella pneumoniae*.Antimicrobial activity was evaluated using colony-forming unit (CFU)-based $\log_{10}$ reduction assays for both planktonic cells and 30 h mature biofilms, employing an MBEC-based model. Bacteriophage activity and stability were assessed using spot assays and plaque-forming unit (PFU/mL) quantification over time in the presence of antimicrobial agents. Additionally, physicochemical properties of honey and propolis, including pH and total phenolic content (TPC), were characterized to support mechanistic interpretation of antimicrobial effects.Results demonstrated that Manuka honey exhibited the strongest antimicrobial and antibiofilm activity among all treatments, achieving near-complete eradication of both planktonic and biofilm-associated bacteria at higher concentrations (50% w/v). Raw honey displayed moderate, concentration-dependent activity, while propolis showed strong antibacterial effects, particularly against *K. pneumoniae*, indicating species-dependent susceptibility. Notably, honey exhibited enhanced efficacy against biofilms compared to planktonic cells, suggesting a mechanism involving disruption of the extracellular matrix independent of bacterial growth phase. In contrast, propolis demonstrated greater activity against planktonic bacteria, consistent with its action on metabolically active cells.However, the combination of bacteriophages with honey, propolis, or ciprofloxacin resulted in reduced antimicrobial efficacy compared to individual treatments. While bacteriophages alone exhibited limited antibiofilm activity, particularly against *E. coli*, their addition to antimicrobial treatments consistently decreased overall bacterial reduction. Phage stability assays revealed that although phages remained viable under most conditions, higher antimicrobial concentrations and prolonged exposure led to reduced infectivity, particularly in the presence of 50% Manuka honey, where no detectable phage activity remained after 16 h. These findings suggest that antimicrobial-induced stress, reduced host availability, or direct physicochemical interference may limit phage replication and adsorption, resulting in antagonistic interactions.This study highlights the strong independent antimicrobial potential of honey and propolis while demonstrating that their combination with bacteriophages may not always produce synergistic effects. Instead, treatment outcomes appear highly dependent on antimicrobial type, concentration, and exposure conditions. These findings underscore the importance of optimizing combination strategies and provide valuable insight into the complex interactions between natural antimicrobial agents and bacteriophage therapy in the context of MDR biofilm eradication.

Gen abstract type

Poster

Section Type

I&I

364 - Engineering *Saccharomyces boulardii* for the expression and secretion of recombinant nanobodies to neutralize cholera toxin

Karla Cristina Cruz[1], David Stuart[1]

[1]University of Alberta

Cholera is a severe diarrheal infection caused by *Vibrio cholerae,* mediated by the secretion of its main virulence factor, cholera toxin (CT). The toxin's B subunit (CTB) binds to the GM1 ganglioside receptor on intestinal epithelial cells, facilitating the endocytosis of the catalytic A subunit (CTA) and subsequent disruption of host signaling pathways. Although antibiotics and vaccines are available, antibiotic resistance and limitations in vaccine access highlight the need for alternative therapeutic strategies.

This study aims to develop a new antimicrobial treatment by engineering the yeast probiotic, *Saccharomyces boulardii* to express and secrete recombinant nanobodies that target cholera toxin and reduce its activity.

To this end, we investigated nanobody (Nb)-based targeting of CTB as a strategy to reduce toxin activity. CTB-binding nanobodies were previously identified through yeast surface display and iterative cell sorting selections, yielding candidates with strong binding affinities to CTB. However, functional evaluation using competitive GM1 ELISA demonstrated that these monomeric Nbs showed limited ability to block CTB-GM1 interactions. To improve inhibitory potential, we designed homologous and heterologous dimeric Nb constructs to enhance avidity and increase the likelihood of engaging the CTB-GM1 binding interface. Molecular modeling approaches were used to guide candidate selection, prioritizing variants predicted to target residues near the GM1 binding pocket of CTB. Selected constructs were cloned into a protein expression vector for subsequent functional testing.

Ongoing work focuses on evaluating the inhibitory performance of these engineered Nbs using competitive GM1 ELISA to quantify inhibition of CTB-GM1 binding. Additional characterization using mass spectrometry (ESI-LC/MS) will further evaluate whether multimerization and rational design can improve the ability of Nbs to interfere with toxin-receptor interactions.

In parallel, to support future microbial delivery, Nb-secreting integrating plasmids were introduced into engineered *Saccharomyces cerevisiae* strains, including protease-deficient mutants and strains expressing multiple copies of chaperones, to identify variants that enhance recombinant Nb secretion.

Overall, this work highlights the importance of integrating binding and functional assays in biologic development. It further demonstrates the potential of engineered nanobodies and yeast-based expression systems as a modular strategy to target bacterial toxins. While *S. cerevisiae* is used here as a tractable chassis for optimization, these efforts provide a foundation for developing a probiotic delivery platform in *Saccharomyces boulardii* for therapeutic applications against *V. cholerae* infections.

Gen abstract type Poster

Section Type I&I

381 - Synergistic Phage and Depolymerase Therapy Overcomes Capsular-Mediated Resistance in Multidrug-Resistant Acinetobacter baumannii

Greg Beaudoin[1], Serena Lam[1], Bryan Schoch[1], Yuliya Martynova[1], Mélanie Arbour[1], Frank St-Michael[1], Wangxue Chen[1], Danielle Peters[1]

[1]National Research Council Canada

Acinetobacter baumannii, a World Health Organization priority-one pathogen, poses a major global health threat due to its extreme antibiotic resistance and virulence. Its thick capsular polysaccharide (CPS) layer shields the bacterium from immune defenses and antibiotics, while also blocking infection by many phages. However, certain specialist phages have evolved to require the CPS for infection and encode capsule-degrading depolymerases (Dpols) to remove the polymer. Leveraging this natural mechanism, we explored whether recombinant Dpols could be used independently or in combination with unrelated phages to strip away the capsule and restore phage susceptibility.

We evaluated the therapeutic potential and synergy of phage derived Dpols with lytic phages against *A. baumannii* strains in vitro. Seven T4 like phages and six recombinant Dpols, derived from distinct phages, were tested in combination against multiple *Acinetobacter* strains. Synergy assays revealed that adding low concentrations of Dpols substantially enhanced phage mediated bacterial suppression and phage amplification compared with phage only controls. Several Dpol-phage pairs also expanded host range without genetic modification, demonstrating strong cooperative activity and adaptability.

To explore translational potential, a pilot in vivo study was conducted using a highly virulent *A. baumannii* strain. Mice received either PBS (untreated control), phage DLP3 alone, Dpol B5 alone, or the combined treatment. Animals treated with DLP3 alone exhibited transient improvement, but some succumbed to infection and the group showed significant clinical symptoms until recovery. Dpol treated mice also lost weight but recovered gradually over time. In contrast, animals receiving the combination showed no clinical signs and survived the full observation period. These findings indicate that while each single agent alone provided partial benefit, their combination markedly enhanced protection and mitigated disease severity in vivo.

Together, these results reveal a cooperative mechanism in which recombinant Dpols remove the capsular polysaccharide barrier that normally blocks phage infection. By degrading the CPS, Dpols expose surface receptors required for phage attachment, enabling productive infection by phages that would otherwise be excluded. The resulting enhancement in phage activity and full protection in vivo underscore the potential of this cross-phage enzyme-phage combination as a powerful antimicrobial strategy.

This work establishes a foundation for next generation antimicrobial development, where phages and Dpols are strategically paired to breach bacterial defenses and improve treatment outcomes. Beyond *A. baumannii*, this adaptable platform could be extended to other encapsulated pathogens, offering a versatile, precision-based approach to combat multidrug-resistant infections.

Gen abstract type

Poster

Section Type

I&I

385 - Comparison of Biofire Film Array Blood Culture ID Panel with Automated Vitek System for Identification of Pathogens and Antimicrobial Resistant Genes in Cancer Patients

Sanjay Biswas[1], Gaurav Salunke[1], Pradnya Samant[1]

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Introduction: Bloodstream infection (BSI) due to multidrug-resistant Gram-negative bacilli is a serious global health problem which has a major impact on severely immunosuppressed neutropenic haematological patients. In this patient population, BSI usually occurs in the setting of febrile neutropenia and is associated with significant mortality. In this scenario, early appropriate empirical antimicrobial therapy is a crucial determinant of improved outcomes. Furthermore, reducing unnecessary antimicrobial usage and avoiding the overuse of broad-spectrum antibiotics is important in the battle against antibiotic resistance and also with regard to containing healthcare costs.

Blood culture remains the gold standard technique for identifying the pathogens responsible for BSI. This approach includes culture-based methods for pathogen identification and antimicrobial drug susceptibility testing. The Film Array Blood Culture Identification 2 panel is a fully automated microbiological diagnostic assay based on nested multiplex PCR analysis that can detect 33 pathogens and 10 antimicrobial resistance genes.

Objectives: This study aimed to assess the efficacy of BCID2 in identifying microorganisms and AMR genes directly from positive blood cultures and to determine its impact on turn around time compared with automated VITEK system for identification of pathogens and resistant genes.

Methods: A total of 35506 samples from 9830 patients were received in the Dept of Microbiology in 2025. All the blood samples were incubated in automated blood culture system for 5 days. Identification and antibiotic susceptibility testing were performed on VITEK-2 system. BCID2 testing was performed, only on request of clinicians, as per manufacturers guidelines.

Results: 2095 blood samples showed growth of pathogens. 104 samples were processed for BCID2 panels. E.coli was most commonest isolate followed by Klebsiella pneumoniae, Neisseria meningitidis , Staphylococcus spp and Pseudomonas aeruginosa . CTX-M with NDM was the most common resistant genotype detected followed by CTX-M with NDM and OXA- 48 like, NDM with OXA-48 like, NDM only and CTX-M with OXA-48like, CTX-M only and OXA- 48 like only. Concordance in identification of organisms was seen in 88% isolates and concordance in resistant genotype and phenotypic sensitivity was seen in 91.7% isolates. Turn around time for BCID was 2 hours whereas VITEK-2 took 2-3 days on an average.

Conclusion: In conclusion, BCID2 showed good performance for the detection of on-panel targets including bloodstream pathogens and antimicrobial resistance determinants, making the assay a promising tool to implement in clinical practice. Nonetheless, in the clinical scenario, the use of molecular methods for the detection of antimicrobial resistance should always be interpreted carefully when used for clinical decision making, due to the complex interaction between genetic determinants and phenotypic profiles.

BCID 2 provided an edge over conventional diagnostics in terms of ease of performing and timing of reporting results. Also, decrease in turnaround time and detection of antibiotic resistance gene may result in early initiation of appropriate antimicrobial therapy and shorter hospital stay.

Gen abstract type Poster

Section Type I&I

389 - Genetic determinants of phage - host specificity in uropathogenic *Escherichia coli*

Lina Siddiqi[1], Karen Maxwell[2], Gregory German[1]

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Uropathogenic *Escherichia coli* (UPEC) is the leading cause of urinary tract infections (UTIs), and is increasingly antimicrobial-resistant, limiting treatment options. Phage therapy, using viruses to kill bacteria, offer a complement to antibiotics in combatting multidrug-resistant infections. Effective uses of phage therapy depend on understanding the bacterial host factors that determine susceptibility. As phage infection depends on the presence and accessibility of specific bacterial surface receptors, alterations of these targets can prevent successful phage attachment and replication. Despite increasing interest in bacteriophage therapy, bacterial determinants governing host specificity in UPEC strains remain poorly defined.

Our objective is to identify host factors required for productive infection, and to assess whether resistance-conferring mutations in these factors impose fitness costs/benefits for bacterial hosts. We hypothesize that genetic variation in UPEC surface components can alter susceptibility to phage infection and, with further study, lead to measurable fitness trade-offs.

Phage susceptibility with 70 clinical UPEC isolates was assessed using spot assays with serial dilutions of 48 diverse phages. This allowed for categorization of isolates and phages based on infection patterns and selection of phage-resistant mutants, which were validated to confirm stable resistance phenotypes, providing a foundation for identifying genetic changes associated with resistance. Whole-genome sequencing and annotation of wild-type isolates identified prophages, antibiotic resistance genes, and phage defence systems, which were all analyzed for their role in resistance.

Among 70 clinical UPEC isolates from The Hospital for Sick Children, we observed substantial variability in susceptibility to 48 phages, reflecting the complexity of phage-host interactions in a clinical environment. From this, we created 7 UTI susceptibility groups based on 4 phage infection phenotypes, complete clearing, hazy, hazy with clear ring, or no lysis. Resistant mutants were isolated across UPEC strains for comparison with wild-type counterparts, alongside planned fitness cost analyses.

Genome annotations of wild-type isolates revealed diversity in genetic determinants of resistance. For instance, variation was observed in prophage content (2-5 genes), resistance genes (54-57, mainly efflux pumps), and phage defence systems (8-16), suggesting that genomic differences contribute to susceptibility profiles, which showed variable alignment with infection outcomes across the panel of phages. Strain UTI 107 (3 prophages) exhibited the highest susceptibility (30/48 phages able to infect/lyse), while strains such as UTI197 (18/48 phages) and UTI114 (21/48 phages) showed lessened susceptibility despite similar prophage and resistance gene loads. Notably, variation in defence system content within the same UPEC sequence-type (UTI 114 and 107 with 14 vs 16 systems, respectively) did not predict susceptibility patterns. These preliminary findings suggest that phage infection outcomes in UPEC is shaped by a combination of genomic features.

This data demonstrates variation in phage susceptibility and confirms isolation of stable resistant mutants. Resistance may carry fitness costs affecting growth, virulence, or antibiotic susceptibility, and ongoing work will define these trade-offs, building on prior studies that have demonstrated such fitness consequences. Ultimately, this project will provide insights into the bacterial determinants of phage susceptibility in a clinically relevant context, supporting both selection and optimization of phage set earmarked for local phage therapy production.

Gen abstract type Poster **Section Type** I&I

394 - Rhomboid protease GlpG regulates type 1 pili quality control and virulence in pathogenic *E. coli*

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With the rise in antimicrobial resistance, understanding the virulence factors utilized by pathogenic *Escherichia coli* is essential for the development of alternative therapeutics. While previous work has shown that disruption of the *E. coli* rhomboid protease GlpG leads to defects in bacterial colonization, our group has provided mechanistic insight into GlpG function and the loss of bacterial fitness. We show GlpG is essential for the assembly of type 1 pili (T1P), a virulence factor required for the initial attachment and subsequent colonization of eukaryotic cells. Our data demonstrates that GlpG plays a key role in protein quality control of FimA, the structural subunits of T1P, and when GlpG is catalytically inactive, uropathogenic *E. coli* lack the T1P that is heavily utilized during host infection. Since pili are critical for biofilm formation and bacterial persistence, we show the absence of GlpG proteolytic activity also reduces the production of biofilm by uropathogenic *E. coli*. Working towards new potential antimicrobial targets for treating infections, we show that biofilm formation is indeed hampered by GlpG inhibition. Together, this work establishes GlpG as a regulator of virulence-associated structures, highlights its potential as a target for anti-virulence therapeutics and alters the paradigm for GlpG proteolysis, previously implicated in the cleavage of only membrane embedded substrates.

Gen abstract type

Poster

Section Type

I&I

401 - An Emerging One Health Threat: Detection of Multidrug-Resistant Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Feacal and Skin Samples of Cattle in Southeastern Nigeria.

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Background and Objectives: Within the One Health framework, the occurrence of livestock-associated methicillin-resistant Staphylococcus aureus (MRSA) represents an extra burden of antimicrobial resistance spanning beyond the community and healthcare settings increasing risks at the human-animal-environment continuum. Livestock production systems in developing countries of sub-Saharan Africa, including Nigeria, are characterized by high misuse/abuse of antimicrobials which promotes the emergence and transmission of resistant and potentially virulent bacteria. This research focused on the detection of multidrug-resistant methicillin-resistant Staphylococcus aureus (MRSA) in faecal and skin samples of cattle bred in Southeastern Nigeria. Methods: A total of seventy-five samples including cattle faeces (n = 35) and cattle skin swabs (n = 40) were collected from cattle using sterile cotton swabs. Staphylococcus aureus was identified using standard morphological and biochemical characteristics. Methicillin-resistant Staphylococcus aureus (MRSA) detection and antibiotic susceptibility testing were done using cefoxitin (30µg) and seven different antibiotics respectively using the Kirby-Bauer disc diffusion method in line with Clinical and Laboratory Standards Institute (CLSI) guidelines. Results: Out of the 75 samples analyzed, Staphylococcus aureus was isolated from 34.3% in faeces and 60% on skin while 20% and 25% MRSA isolates were identified from the cattle faeces and skin respectively. Faecal MRSA isolates showed high resistance to linezolid and amoxicillin clavulanate (100%), ceftriaxone (91.7%), imipenem (83.3%) and amikacin (66.7%) but exhibited high sensitivity to levofloxacin (100%) and azithromycin (91.7%). For the cattle skin, the MRSA isolates demonstrated high susceptibility to amikacin and imipenem (100%), levofloxacin and ceftriaxone (95.8%), and moderate resistance to linezolid (91.6%). Multiple antibiotic resistance index (MARI) was 0.54 for faecal isolates and 0.29 for skin isolates. Conclusion and Recommendations: The detection of multidrug-resistant MRSA in cattle, especially in faeces, highlights significant zoonotic transmission risks within the One Health nexus. Therefore, antimicrobial stewardship, enhanced hygiene and surveillance across human and animal health systems are vital to curb the spread of MRSA.

Keywords: Emerging, One Health, Multidrug Resistant, MRSA, Cattle

Gen abstract type

Poster

Section Type

I&I

402 - Carriage of Multidrug Resistant Bacterial Species on Goat Skin and in the Nasal Cavity: A One Health Assessment of Zoonotic Risk

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Background and Objectives: Goat or domestic goat (*Capra hircus*), member of the animal family Bovidae and the tribe Caprini and a domesticated species of goat-antelope is typically kept as livestock. In developing countries like Nigeria, most times, there is a close association between humans and these animals which promotes the emergence and zoonotic transmission of resistant and potentially virulent bacteria. In alignment with one health perspective, this research focused on the colonization of multidrug resistant bacterial species in goats bred in Abakaliki, Eastern Nigeria and evaluated the possible zoonotic transmission risk in relation to human health. Methods: A total of 165 samples (110-skin and 55-nares) were collected from goats using sterile cotton swabs. The morphological and biochemical characteristics of the bacterial species isolated from the skin and nasal samples of the goats were examined. Methicillin-resistant *Staphylococcus aureus* MRSA) detection and antibiotic susceptibility testing were done using cefoxitin (30µg) and seven different antibiotics respectively for one hundred and ten (110) randomly selected bacterial isolates using Kirby-Bauer disc diffusion method. Results: Out of one hundred and sixty five (165) samples collected, results showed isolation rate of Staph species 132(43.6%) with the highest percentage frequency of occurrence, then *Escherichia coli* 66(21.8%) *Shigella* species 39(12.7%), *Klebsiella* species 44(14.5%) and *Salmonella* species 22(7.3%). Further studies revealed that all the randomly selected *S. aureus* (100%) were MRSA. Bacterial isolates were highly resistant to amoxicillin-clavulanic acid 110(100%), linezolid 100(90.9%), levofloxacin 83(75.45%) and ceftriaxone 69(62.72%). Moderate susceptibility was observed for imipenem 78(70.90%), and amikacin 77(70.00%). Cummulative average MARI of the isolates was 0.52 with MARI values ≥ 0.2. Conclusion and Recommendations: The results of this study reveal that the carriage of zoonotic bacteria including MRSA on goat as a result of clonal spread and multidrug resistance characteristics highlights a significant One Health concern at the human-animal-environment interface. This study suggests that the awareness of veterinarians of the most effective and prudent use of antibiotics when combating bacterial infections combined with standard hygiene protocols are the primary methods for controlling the spread of these resistant bacteria.

Keywords: Multidrug Resistant, Skin, Nasal, One Health, Zoonotic Risk

Gen abstract type

Poster

Section Type

I&I

MOLECULAR GENETICS & CELL MICROBIOLOGY (MGCM) ABSTRACTS/ RÉSUMÉS DE GÉNÉTIQUE MOLÉCULAIRE ET DE MICROBIOLOGIE CELLULAIRE (MGCM)

2 - Low-Level Tetracycline Exposure Promotes Long-Term *Salmonella* Persistence and Plasmid-Mediated Gene Transfer in a Dysbiotic Murine Gut Model

Amer Abdelgany[1, 2], Tamara Morrill[2], Calvin Lau[3], Catherine Carrillo[3], Alex Wong[1], Jiewen Guan[2]

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Antimicrobial resistance (AMR) is a major One Health challenge driven by the persistence of resistant pathogens and the dissemination of plasmid-borne resistance genes within complex microbial ecosystems. The gastrointestinal tract is a major site of horizontal gene transfer (HGT), particularly under antibiotic-induced dysbiosis. However, the long-term impacts of low-level tetracycline exposure on *Salmonella* persistence and plasmid transfer dynamics remain poorly understood.

Using a murine dysbiosis model, animals were treated with ampicillin to disrupt the gut microbiome and subsequently colonized with multidrug-resistant *Salmonella* carrying a conjugative AMR plasmid. Mice were then exposed to a tetracycline gradient spanning environmentally relevant and subclinical exposure levels. Longitudinal fecal sampling was performed to assess pathogen persistence and plasmid transfer using culture-based methods and qPCR. Microbiome changes were evaluated by 16S rRNA gene sequencing. Representative transconjugant isolates were additionally confirmed by sequencing to validate plasmid acquisition and identify recipient lineages across exposure conditions.

Culture-based analyses confirmed that *Salmonella* persisted across all tetracycline exposure conditions, with the highest persistence observed under the highest tetracycline exposure group. Plasmid transfer to commensal Enterobacteriaceae was detected in all tetracycline-exposed groups, with distinct transconjugant genera recovered across exposure levels, suggesting dose-dependent shifts in recipient populations. Transfer to an external recipient strain was additionally detected under the highest exposure condition, supporting enhanced plasmid mobility under increased tetracycline pressure. qPCR further confirmed plasmid gene detection across all tetracycline-exposed groups, consistent with sustained plasmid maintenance and dissemination. Microbiome profiling revealed that the highest tetracycline exposure group exhibited reduced alpha diversity and increased Proteobacteria relative abundance, consistent with antibiotic-driven dysbiosis and ecological conditions favorable for HGT.

These findings demonstrate that low-level tetracycline exposure within environmentally relevant and subclinical ranges can promote long-term *Salmonella* persistence and facilitate plasmid-mediated AMR gene transfer in the gut. This work highlights the potential role of antimicrobial residues in sustaining AMR reservoirs and accelerating resistance dissemination, reinforcing the importance of incorporating low-dose antibiotic exposures into One Health AMR risk assessments.

Gen abstract type Poster

Section Type MGCM

5 - Dose-dependent antifungal tolerance in *Candidozyma auris*

Samira Rasouli Koohi[1], Clare Maristela Galon[1], Daniel Charlebois[1, 2]

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Fungal pathogens cause over 1.6 million deaths annually across the globe and make a major contribution to the “silent pandemic” or antimicrobial resistance. Antifungal resistance poses unique challenges due to limited antifungal drug classes, similarities to “higher” eukaryotes, and climate change. Antifungal tolerance is a recently discovered phenomenon whereby pathogenic fungi, including the multidrug-resistant pathogenic yeast *Candidozyma auris* (formerly *Candida auris*), grow slowly above minimum inhibitory drug concentrations [1]. We combine physics-based modelling with microbiology experiments to quantitatively investigate the emergence of tolerance to all three major classes of antifungal drugs in *C. auris* [2]. Specifically, we combine drug diffusion modelling with disk diffusion assays to determine the concentrations at which tolerant colonies emerge. This study advances antimicrobial resistance research by demonstrating that antifungal drug tolerance is a dose-dependent phenomenon and by providing a method to quantify tolerance in fungal pathogens.

Abstract category choice

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Section Type

MGCM

8 - *Pseudomonas aeruginosa* bacteriophage discovery and characterization using the SURFAR platform

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[1]McMaster University

Background: *Pseudomonas aeruginosa* infections are increasingly resistant to available antibiotics. In addition, the WHO has listed carbapenem resistant *P. aeruginosa* as a high priority pathogen. Novel approaches such as the use of bacteriophages (phages), viruses that specifically infect bacteria, have been increasingly explored. While novel phages are constantly being reported on, there is no standard for their characterization. Phage receptors are suggested to be strong predictors of phage susceptibility, highlighting the importance of systematically defining receptor usage.

Results: With the goal of expanding the diversity of *P. aeruginosa* phages, we present the SURFAce Receptor or SURFAR platform, a set of mutant and recombinant strains for identification of new phages that bind non-canonical surface structures or novel receptor combinations, including those that may be more highly expressed during infection. Using this platform, we identified two novel *P. aeruginosa* targeting phages that use combinations of canonical receptors. These phages can also infect multiple strains of *P. aeruginosa* including carbapenem resistant clinical isolates of the pathogen.

Significance: Phage therapy is often administered as cocktails (multiple phages) consisting of phages that target different receptors. Therefore, identification of a novel phage that can use multiple phage receptors may circumvent the need to purify and produce multiple phages- a laborious process. Our platform and approach are phage receptor focused as receptor characterization enables the use of phage steering: the strategy of using carefully chosen phages to intentionally select for resistant strains that have lost specific traits. Steering focuses on controlling the outcome and downstream effects of bacterial resistance, rather than simple elimination of bacteria through killing. Many phage receptors are key virulence factors or outer membrane proteins involved in antibiotic efflux. Therefore, using phages to select for loss of these components can reduce bacterial fitness and increase antibiotic susceptibility, which are ideal clinical outcomes.

Gen abstract type

Poster

Section Type

MGCM

20 - Discovery and characterization of novel anti-phage defense systems in *Streptococcus thermophilus*

Christina Rossignol-Garon[1], Sylvain Moineau[1], Audrey Leprince[1, 2]

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Streptococcus thermophilus, a lactic acid bacterium essential for the production of yogurt and certain cheeses, plays a central role in the Canadian dairy industry. Due to its rapid growth in milk and strong acidification capacity, it is widely used for industrial fermentations. However, this species is particularly vulnerable to bacteriophages, which are a major cause of fermentation failures and can lead to reduced quality of fermented dairy products. To reduce these risks, the industry notably relies on naturally phage-resistant strains, particularly through CRISPR-Cas systems. However, some phages have developed escape strategies, including the accumulation of mutations or deletions in targeted regions, as well as the production of anti-CRISPR (ACR) proteins capable of neutralizing this defense. This evolutionary arms race between phages and bacteria highlights the need to identify new resistance mechanisms in *S. thermophilus.*

This project aims to: (1) identify and test new candidate defense systems in *S. thermophilus* to evaluate their effectiveness against a broad range of phages, and (2) characterize the mode of action of the most promising system using microbiological, bioinformatic, and biochemical approaches. Candidate systems were selected through genomic analyses targeting defense-associated genomic islands, which are recognized as important reservoirs of bacterial antiviral systems. These regions also contain many genes encoding proteins of unknown function, some of which may correspond to novel defense mechanisms.

The selected candidates were introduced into industrial phage-sensitive *S. thermophilus* strains using an expression vector, then evaluated by spot assays against 16 phages isolated from dairy samples and representing the five viral genera known to infect this bacterial species. Some of the phages used encode an ACR protein. This strategy also made it possible to assess whether the tested systems could protect the bacterium against phages infecting five industrial *S. thermophilus* strains. To date, a first anti-phage system composed of two genes has already been identified. This system is currently undergoing in-depth characterization to define its spectrum of activity and mode of action.

Overall, this project will contribute to expanding knowledge of bacterial antiviral strategies and of the *S. thermophilus* defensome. In the longer term, it may benefit the dairy industry through the combination of some of these mechanisms within a single strain by natural competence, in order to limit the number of phages able to overcome defense systems. In this way, it could support the development of more robust industrial strains against phage infection and thereby contribute to the stability and quality of dairy fermentations.

Gen abstract type Poster

Section Type MGCM

21 - A reverse vaccinology approach to identifying novel targets for protein-based vaccines against *Streptococcus pneumoniae*

Bernice Ramos[1, 2], Bhavjinder K Dhillon[3], Alyssa Golden[4], James D Kellner[5], Nicholas Brousseau[6], Manish Sadarangani[3, 7]

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Introduction. Despite the use of pneumococcal conjugate vaccines (PCVs), *S. pneumoniae* remains a leading cause of global morbidity and mortality, globally. While PCVs are highly effective at preventing IPD and transmission, they cover only up to 21 out of the 100 disease-causing serotypes. Protein-based vaccines could overcome this limitation by targeting antigens present across all serotypes. However, amino acid (AA) variation at the protein level may reduce vaccine effectiveness by enabling escape from vaccine-induced immunity. We therefore assessed AA variation of vaccine candidates previously studied in clinical trials, such as pneumolysin (Ply), pneumococcal histidine triad protein D (PhtD), pneumococcal surface protein A (PspA), and pneumococcal surface antigen A (PsaA), and identified novel candidates, prioritizing cell wall proteins with low AA variation.

Methods. The National Microbiology Laboratory provided whole-genome sequencing data on 998 IPD isolates from children and adults from across Canada, except for Alberta, from 2018-2020. Genomes were annotated using Bakta using the *S. pneumoniae* R6 genome as a reference. We used the core genome (core=80%) produced by Roary to identify highly conserved proteins alongside PhtD, Ply, PspA, and PsaA. PSORTb *v3.0.2* was used to predict cellular localization, prioritizing cell wall proteins (CW). AA variation was assessed using Block Mapping Gathering Entropy (BMGE), with highly conserved regions having scores closer to 0 and highly variable regions close to 1. Proteins with the lowest BMGE scores were prioritized. Cross-reactivity against the human genome was inspected using BLAST sequence similarity. Transmembrane prediction was done using DeepTMHMM *v1.0.67* to identify regions most accessible to the immune system. Adhesion was predicted using SPAAN.

Results. Of the core genome, 20 (1.2%) were CW proteins. Previous vaccine candidates were present in 63% (PhtD) to 99% (Ply) of all IPD isolates. Of the CW proteins, presence ranged from 92% (CW_15) to 100% (CW_1, CW_2, CW_3, CW_4, CW_5, CW_6_, CW_7). Five proteins had significant similarity to human proteins and therefore were not considered for further evaluation. Nine were adhesins, and two had transmembrane domains. PhtD and PspA showed high amino acid variation, and there was no distinct region of conservation, while Ply and PsaA showed minimal variation. The most conserved proteins CW_2 (peaks at 0.18, 0.13), CW_10 (peaks at 0. 03), and CW_8 (peaks at 0.22, 0.2) were selected as potential novel vaccine candidates due to their low AA variation. Proteins CW_13 (peaks at 0.54, 0.49), CW_11 (peaks at 0.33, 0.32), and CW_1 (peaks at 0.21, 0.20) were shown as examples of proteins displaying high AA variation across the whole protein and thus less likely to be broadly protective vaccine candidates.

Conclusions. Protein vaccine development may benefit from prioritizing highly conserved proteins with minimal amino acid variation, such as PsaA or our novel candidates (CW_2, CW_10, CW_8), or by including multiple protein variants or antigens.

Gen abstract type Poster

Section Type MGCM

22 - Phylogenetic analysis of *Streptococcus pneumoniae* isolates from cases of invasive pneumococcal disease in Canada, 2018-2020

Bernice Ramos[1,2], Bhavjinder K Dhillon[3], Alyssa Golden[4], James D Kellner[5], Nicholas Brousseau[6], Manish Sadarangani[3,7]

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Introduction. *Streptococcus pneumoniae* remains the leading cause of lower respiratory tract infections globally. *S. pneumoniae* colonizes the nose and throat, usually asymptomatically, often referred to as carriage. Further spread to sterile body sites, such as the blood and cerebrospinal fluid, can cause invasive pneumococcal disease (IPD), including sepsis and meningitis, respectively. The capsular polysaccharide (CPS), a major virulence factor, is structurally and serologically unique to each serotype. The CPS is exploited by pneumococcal conjugate vaccines (PCVs), and PCVs are included in immunization programs for children <2 years and adults. PCVs are highly effective at reducing IPD and colonization in those directly immunized, preventing carriage and transmission, thus indirectly protecting unimmunized groups. However, the removal of VTs from the population allows non-vaccine serotypes (NVTs) to emerge, replacing the disease burden. Furthermore, some serotypes in PCVs (e.g., 3, 19F, 19A) continue to cause IPD for reasons that remain unclear. Serotype alone cannot fully explain these dynamics, highlighting the need to examine the genetics and phylogeny of *S. pneumoniae,* including sequence types (STs), global pneumococcal sequence clusters (GPSCs), and multi-drug resistance (MDR). This study characterized the genomic features of IPD isolates in Canada.

Methods. The National Microbiology Laboratory (NML) provided whole genome sequencing data on 998 IPD isolates from children and adults from across Canada, except for Alberta, from 2018-2020. Genomes were annotated with Bakta*,* using the *S. pneumoniae* R6 genome as a reference. Phylogeny was constructed from the core genome (core=80%) with Roary and RAxML-ng, and recombination-adjusted with ClonalFrameML. The final tree was then annotated with MicroReact. STs and GPSCs were called using PubMLST and PopPUNK, respectively. The NML WGS Analysis and Detection of Molecular Markers (WADE) pipeline was used to predict antibiotic resistance genes to infer antimicrobial resistance (AMR). Multi-drug resistance (MDR) was defined as being resistant to ≥3 antibiotic classes.

Results. The core genome comprised 1674 genes (22%) of the pangenome (n=7500). A total of 80 GPSCs and 196 sequence types (STs) were identified. Clear associations of GPSCs with STs and serotypes were observed: GPSC12 (ST180) included serotype 3, GPSC3 (ST62, ST100, ST53) included serotypes 33F, 11A, and 8, GPSC19 (ST433) included serotype 22F, GPSC27 (ST695) included serotypes 4 and 19A, and GPSC6 (ST3811, ST162) included serotypes 15A and 24F. AMR was most common to azithromycin (27%), some to sulfamethoxazole (14%), and penicillin (13%). Resistance to ceftriaxone was uncommon (4%). A few isolates that were not part of a major GPSC/ST displayed penicillin resistance. Overall, 117/994 (12%) of isolates exhibited MDR; these were mostly associated with GPSC904;9 (serotype 15A), GPSC59 (ST558; serotype 29, 35B), GPSC5 (ST338; serotype 23A), GPSC1 (ST320; serotype 19A), and other non-major GPSCs/STs.

Conclusion. Our findings showcase the genetic lineages driving IPD in Canada and their resistance profiles, providing evidence to refine future vaccine serotype targets and support surveillance efforts focused on emerging and MDR pneumococcal clusters.

Gen abstract type Poster **Section Type** MGCM

23 - Characterization of a Novel Type VI Secretion System Regulator in *Pseudomonas aeruginosa*

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Bacteria live in crowded environments where survival depends on their ability to compete for space and nutrients. To gain an advantage, some Gram-negative bacteria are equipped with a specialized protein secretion apparatus called the Type VI Secretion System (T6SS). *Pseudomonas aeruginosa*, a widespread and opportunistic bacterium, encodes multiple T6SSs that are tightly regulated to avoid wasting energy when competitors are absent.

While three *P. aeruginosa* T6SSs (H1, H2, and H3) have been well studied, a recently discovered fourth system, the H4-T6SS, remains largely unexplored. This system is rare, present in only about one percent of strains, and is encoded on a transposable element. Notably, the H4-T6SS gene cluster encodes an uncharacterized regulatory protein, raising questions about how this system is activated.

My project aims to define how the H4-T6SS is regulated. Using bioinformatics analyses and bacterial secretion assays, I identified that this regulatory protein functions as a sigma factor activator, a family of proteins that regulate gene expression through interactions with the alternative sigma factor σ^{54}. I named this protein Sfa4, for **s**igma **f**actor **a**ctivator of the H**4**-T6SS. I found that Sfa4 positively regulates H4-T6SS gene expression, while secretion by the system is controlled at a post-translational level, revealing an additional and unexpected layer of regulation.

Overall, my project defines how a novel, horizontally acquired T6SS is regulated in *P. aeruginosa*. These findings show how foreign genes can be repurposed to enhance bacterial fitness and provide insight into how *P. aeruginosa* continues to evolve new strategies for bacterial competition and infection.

Gen abstract type

Poster

Section Type

MGCM

29 - Carbapenemase-Producing Enterobacteriaceae Plasmid Host Assignment Through Epigenetic Pattern Matching via Methylation-aware Nanopore Sequencing

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Carbapenems are considered antibiotics of last resort for drug-resistant Gram-negative bacterial infections. Some hospitalized patients are asymptomatically colonized by CPEs (Carbapenemase-producing *Enterobacteriaceae*) that can cause invasive infections if they escape the gut. Mutations in carbapenemase genes have led to unexpected resistance to novel antibiotics or difficulties in detection using traditional diagnostic methods. Resistance to carbapenem antibiotics is primarily encoded by ß-lactamase genes transferred on broad host-range plasmids. As the human gut provides a high density niche for plasmid transfer, plasmids carrying carbapenemase genes can quickly spread resistance to opportunistic pathogens. Many hospitals routinely screen patients for CPE colonization using multiplex CPR, yet there are limits to the number of genes targeted and PCR itself is inherently limited to known resistance genes and variants. While shotgun metagenomics and target enrichment (bait capture) are viable culture-free strategies for recovering bacterial genomes and resistance genes, broad host-range plasmids do not carry host-identifying gene sequences and thus they cannot be associated with the original resistant host.

Instead, epigenetic DNA modifications can be used to link plasmid DNA to a host chromosome. During DNA methylation, methyltransferase enzymes add a methyl group to adenine or cytosine bases. All of the DNA in a bacterial cell, including plasmids, is methylated by the same methyltransferase enzymes, producing a characteristic methylation pattern. This molecular barcode can be used to group DNA molecules and identify plasmid hosts. Bacterial methyltransferases are highly diverse amongst the bacterial kingdom, and can produce distinct patterns even amongst isolates of the same species, making their products suitable for the identification of original plasmid hosts.

We designed MethLinker, a software pipeline for culture-free plasmid host identification, to identify organism-specific epigenetic barcodes that correctly associate plasmids with their original host chromosome. MethLinker identifies, clusters, and visualizes methylated motifs across long-read contigs to link CPE plasmids to a host. Using MethLinker, we successfully linked plasmids to their host chromosomes using a test set of 10 *Enterobacteriaceae* isolated from human stool, and were able to differentiate between strains of the same species based on methylation patterns.

Prior to validating MethLinker with clinical swabs, we will determine the limit of detection for plasmid-host association using mock metagenomic samples. We extracted DNA from an *Enterobacter cloacae* strain carrying an NDM-1 plasmid and spiked it into a ZymoBiomics High Molecular Weight DNA Standard at concentrations ranging from 10^{-6} to 10^{-1}. These concentrations will more accurately reflect carriage of CPEs in clinical data and will help us determine whether pre-sequencing enrichment will be required for clinical validation. Spike-in samples will be sequenced using Nanopore R10.4.1 flow cells before identifying methylation patterns with MethLinker and visualizing clusters to determine the number of plasmid copies required to reliably link our resistance plasmid to its host.

Finally, in partnership with Hamilton Health Sciences, we will extract total DNA from CPE-positive rectal swabs and sequence these to identify carbapenemase-encoding plasmids and associate them with their host chromosome. We will compare the resistant organisms identified using MethLinker to the culture-based results from each swab.

Gen abstract type

Poster

Section Type

MGCM

33 - Determining regulators of production and the molecular mechanism responsible for capsular alpha-glucan export in the *Mycobacterium tuberculosis* complex

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Tuberculosis, caused by members of the *Mycobacterial tuberculosis* complex (MTBC) is the deadliest disease, and affects both humans and animals. Mycobacteria are encapsulated by an extracellular capsule, with the most abundant component being the branched polysaccharide alpha-glucan. Mycobacterial alpha-glucan is synthesized within the cell using the enzymes GlgE to elongate the polysaccharide chain and GlgB to create branching, but the genetic basis and mechanism for how alpha-glucan production is regulated and how the polysaccharide is exported to the capsule remain unknown.

Through an immunoblot assay-based capsular polysaccharide survey of various MTBC species, we discovered that the capsule of *Mycobacterium bovis* AF2122/97, a type strain and causative agent of zoonotic bovine tuberculosis, does not contain alpha-glucan, though components of the carbohydrate inside the *M. bovis* AF2122/97 cell can be detected by the monoclonal antibody used in the immunoblot assay. We also performed iodine vapour staining of MTBC colonies, in which the iodine vapour upon contact with capsular branched polysaccharides like capsular alpha-glucan stains red brown in colour while contact with linear polysaccharides such as intracellular alpha-glucan stains deep blue. In our iodine vapour staining experiments of *M. tuberculosis* H37Rv and *M. bovis* AF2122/97, no staining was observed for *M. bovis* AF2122/97, indicating that *M. bovis* AF2122/97 may be unable to synthesize the full capsular alpha-glucan product within the cell in addition to being unable to export the components of the carbohydrate to the capsule. Our immunoblot assay-based capsular polysaccharide survey also revealed that select external growth conditions, such as hypoxia, affect the presence of capsular alpha-glucan. We aim to explore both the means in which alpha-glucan production is regulated and exported in the MTBC.

Our lab has an ordered library of cosmids containing DNA fragments that encompasses the genome of *M. tuberculosis* H37Rv, an alpha-glucan production and export-competent MTBC. We hypothesize that systematically transforming *M. bovis* AF2122/97 via electroporation with individual cosmids covering the *M. tuberculosis* H37Rv genome, will allow us to determine the genetic basis of the unique phenotype of *M. bovis* AF2122/97. Further analysis of polysaccharide extracts from *M. bovis* AF2122/97 will enable us to identify the characteristics of the carbohydrate components detected within the cell with our immunoblot assay-based capsular polysaccharide survey. Alongside gene expression studies of the enzymes GlgE and GlgB involved in alpha-glucan biosynthesis, this will provide an explanation for the molecular basis responsible for the unique phenotype in *M. bovis* AF2122/97. Additional experiments in which *M. tuberculosis* H37Rv and *M. bovis* AF2122/97 will be grown in varying environmental conditions will shed light on the regulation of capsular alpha-glucan production and export in the MTBC.

Gen abstract type Poster

Section Type MGCM

34 - Modulating Valerate Levels *in vivo*

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The short-chain fatty acids (SCFAs), acetate, propionate and butyrate, are produced in the gut by the resident microbiota and have anti-inflammatory properties. They aid in colonization resistance, provide energy to colonocytes, and modulate the immune system. In addition to the most abundant SCFAs, the microbiota also produces valerate, a 5-carbon fatty acid, though the pathway for its production is not well understood. Our lab recently demonstrated that, under baseline conditions, *Lachnospiraceae,* which are obligate gut anaerobes, don't produce valerate. However, through the reverse β-oxidation pathway, *Lachnospiraceae* can combine acetate and propionate to yield valerate when extracellular SCFAs are present. Recent evidence indicates that valerate is an essential metabolite with its anti-inflammatory properties and inhibition of *C. albicans* and *C. difficile*. This emphasizes the importance of developing strategies to control its production *in vivo*. To optimize the production of SCFA and valerate, we conducted a screening of 65 gut where each isolate was added to a base consortium comprising *Anaerostipes hadrus, Coprococcus comes, Clostridium innocuum,* and *Anaerotignum lactatifermentans,* a high propionate producer. Supernatants were collected, and metabolites were measured through gas chromatography-mass spectrometry. This revealed that certain gut isolates could produce valerate at higher levels when combined with the base consortium. The top five valerate producers identified in the screening were then mixed with the base consortium in all possible combinations to assess if these microbes would have an additive effect on propionate and subsequent valerate production. Based on these findings, a final mix of *Lachnospira [Eubacterium] eligens*, *Eubacterium callanderi*, *C. comes*, *A. hadrus* and *C. innocuum* was selected for *in vivo* applications. *A. lactatifermentans*, *Veillonella dispar* and *Veillonella atypica* were added to increase the overall propionate and therefore the valerate concentrations. These consortia were then gavaged into mice to test whether the trends could replicate *in vivo*. The results demonstrated successful *in vivo* modulation of valerate, although propionate levels peaked and subsequently decreased, thereby influencing valerate levels. A second experiment was conducted incorporating *Bacteroides thetaiotaomicron* into the 8-isolate consortium, given its capacity to produce propionate and its high adaptability in the murine gut. As anticipated, the consortia with *B. thetaiotaomicron* increased the overall propionate levels, thereby increasing valerate, and showed a greater stability across multiple days. Overall, these results demonstrated a successful *in vivo* modulation of valerate, achieving physiological levels. In future work, this current consortium of 9 isolates could be further optimized through the removal of isolates that are non-essential *in vivo* and by adding higher-propionate producers.

Gen abstract type

Poster

Section Type

MGCM

41 - An Efficient, Trigonometry-Based Approach for High-Throughput Whole Genome Alignment

Lindsay Moffatt[1], Matthew Sorbara[1]

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Identifying gut anaerobes that perform beneficial functions is important for live biotherapeutic development. However, inconsistent or changing taxonomy and difficulty delineating bacterial species makes associating functions with specific taxa challenging. Furthermore, the use of cutoffs in quantitative metrics of relatedness, such as ANI or 16S rRNA identity, to define species can separate closely related strains into different taxonomic groups. Synteny-based approaches represent an increasingly feasible alternative given the rising availability of circularized genomes. However, current alignment tools generally work only on linear sequences, causing loss of information when circular topology is flattened to a linear one. Here, we develop an efficient, trigonometry-based approach for genome analysis for circularized genomes, capable of hundreds of genome comparisons/min on a standard system. Leveraging this efficiency, we analyze ~1.5 million pairs of gut anaerobe genomes.

Our approach aligns genomes and detects inversion events. Alignment minimises the distance between pairs of shared, single-copy genes. First, coordinates of each gene on the Cartesian plane are computed from the genome size and the gene start position trigonometrically. Then, Euclidean distance is calculated for each gene pair. One genome is then 'rotated' relative to the other in fixed intervals to find the alignment which minimises these distances. When synteny is well-maintained, lines drawn between gene pairs will be largely parallel to one another, such that a barrel-like shape is formed. Otherwise, these visualizations appear disorganized. The approach ultimately outputs both the binary decision on barrel formation and the visualization. Inversion detection is based on the presence of blocks of genes which, when reflected over their centre, become much closer to their partner on the other genome than in the original aligned state. A binary decision is then output for presence of an inversion.

We found that barrel formation is strikingly dichotomous: most pairs either form clear barrels or are completely disorganized, with a vanishingly small proportion occupying an intermediate state. "Barrel groups"—clusters of genomes which consistently form barrels with one another—often corresponded to species, revealing that species maintain their genomic architecture despite large accessory genomes. However, in some taxa, barrel groups instead segregated a genus into subgroups which each included multiple species. In these cases, barrel groups generally corresponded to known clades within the genus. In addition, we observed that large inversions between otherwise barrel-forming genome pairs are relatively common in gut anaerobes.

We are interested in using this approach to understand how microbial genomes change across time, geography, and lifestyle of their host. To do so, we will assess barrel formation for genome pairs derived from conserved taxa between industrialized and non-industrialized or contemporary and ancient microbiomes. As such genomes are often only available as MAGs, we have developed a modified pipeline which can align draft genomes to circularized ones.

Our results indicate that identifying barrel groups and conserved inversions provides insight into isolate relatedness. This technique thereby holds promise for understanding genomic evolution of microbes and its link to functional divergence of taxa.

Gen abstract type Poster

Section Type MGCM

46 - Differential use of Stickland fermentation substrates by species of Lachnospiraceae alters the metabolic strategy of Clostridioides difficile

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Clostridioides difficile infection (CDI) causes severe infectious colitis and commonly arises following antibiotic treatment. Antibiotics disrupt the gut microbiota and impair colonization resistance (CR), a fundamental function of a diverse microbial community that limits pathogen engraftment. Key mechanisms include nutrient competition, production of inhibitory metabolites, and modulation of host immune responses. Loss of these functions reshapes the intestinal environment in ways that permit pathogen expansion. Current treatments for CDI rely on further antibiotic use, compounding microbiota disruption and increasing the risk of recurrent infection. As such, identifying specific microbial functions that contribute to CR represents a promising strategy for developing therapeutics that restore microbiota function and reduce CDI recurrence.

During early gut colonization, *C. difficile* performs Stickland fermentation, coupling amino acid oxidation and reduction to generate energy. Among a limited set of preferred electron acceptors, proline plays a central role, undergoing conversion from L- to D-proline via proline racemase (prdF), followed by reduction to 5-AV by the proline reductase complex (prdA, prdB). Proline availability within the gut can therefore influence *C. difficile* expansion. Importantly, enteric proline can exist as free proline, hydroxyproline or proline-containing di- and oligopeptides, constituting a diverse nutrient niche. Select members of the dominant *Lachnospiraceae* family also access this niche; however, the extent to which they consume distinct proline sources and how this influences *C. difficile* colonization remains unclear.

Here, we identified a phylogenetically distinct clade of *Lachnospiraceae* encoding a conserved proline reductase operon. As expected, all isolates performed Stickland fermentation in rich media, reducing proline to 5-AV. However, species exhibited distinct proline utilization strategies. While most depleted free proline stoichiometrically, isolates of two species, *Coprococcus comes* and *Sellimonas intestinalis,* increased proline concentrations. Genomic analyses revealed that these species encode an expanded repertoire of proline-targeting peptidases and peptide transport systems but lack canonical free-proline transporters. Accordingly, they failed to use free proline in defined media and instead accessed proline from dipeptides. Other Prd-encoding *Lachnospiraceae* in defined media produced 5-AV from free proline and dipeptides, as well as other proline derivatives such as hydroxyproline. These findings reveal metabolic specialization among *Lachnospiraceae* and suggest that competition for distinct proline sources may contribute to protection from CDI.

To test the functional consequences of these differences, we examined their impact on *C. difficile* growth. Depletion of free proline by select *Lachnospiraceae* eliminated the growth advantage of wild-type *C. difficile* over a Stickland-deficient mutant (DprdB). In contrast, peptide-specialist species increased proline availability and enhanced the wild-type advantage. Together, these findings identify proline metabolism as a strain-dependent mechanism of colonization resistance. They demonstrate how variation in metabolic niche occupation can differentially constrain *C. difficile* expansion and inform the development of microbiota-directed therapies.

Gen abstract type

Poster

Section Type

MGCM

49 - Investigating Genetic Drivers of Multidrug Resistance and Antisense-Mediated Therapeutic Reversal in *Candidozyma auris*

Michael Reich[1, 2, 3], Karolina Czajka[2], Chibike Ijomah[1, 2], Yamamah Al-Jumaili[1, 2], Anu Nair[1, 2, 3], Sujeenthar Tharmalingam[1, 2, 3]

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Candidozyma auris is an emerging multidrug-resistant (MDR) fungal pathogen that poses a significant global health threat. It is classified as a critical-priority fungal pathogen by the World Health Organization and an urgent antimicrobial resistance threat by the U.S. Centers for Disease Control and Prevention. Since its first identification, *C. auris* has been associated with nosocomial outbreaks, high mortality rates, and an exceptional capacity to acquire resistance to multiple classes of antifungal agents. While resistance to azoles, polyenes, and echinocandins has been widely reported, the molecular determinants driving this MDR phenotype remain incompletely characterized, particularly in clinical isolates.

This study aims to (1) characterize the genetic basis of azole, polyene, and echinocandin resistance in a clinical *C. auris* isolate (B12244) and (2) evaluate antisense oligonucleotide (ASO)-based gene silencing as both a functional validation tool and a potential therapeutic strategy. Resistance-associated gene expression and sequence variation will be assessed using RT-qPCR and RNA sequencing, respectively. These data will inform the rational design of ASOs targeting mutant or overexpressed transcripts implicated in antifungal resistance. ASOs will be delivered using lipid-based nanoparticles to facilitate cellular uptake and transcript engagement.

Restoration of antifungal susceptibility will be evaluated through growth-curve-based phenotypic assays, while transcript knockdown efficiency will be quantified by RT-qPCR. Preliminary data indicate that isolate B12244 exhibits marked fluconazole resistance, with minimum inhibitory concentrations approximately five-fold above suggested clinical breakpoints. Collectively, this work addresses a critical gap in *C. auris* pathogenesis by elucidating genetic drivers of MDR and introducing a precision, gene-targeted strategy for therapeutic reversal. By integrating molecular genetics with nanotechnology-based delivery, this study establishes a framework for combating antifungal resistance in a rapidly emerging global pathogen.

Gen abstract type

Poster

Section Type

MGCM

53 - Determining the environmental specificity of *Pseudomonas aeruginosa* competition genes

Kylah Rendell[1], Duncan Fehr[1], Sean Booth[1]

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Limited resources and spatial constraints compel bacteria to engage in constant competition. *Pseudomonas aeruginosa*, an opportunistic pathogen, encodes multiple antibacterial weapon systems within its genome, including the seemingly redundant S-type pyocins. The type strain, PAO1, encodes three S pyocins: S2, S4, and S5. Why *P. aeruginosa* encodes multiple pyocins is unclear, but previous work showed that the environmental iron concentration modulates the effectiveness of S2 and S5, but not S4. A transcriptomic study showed that S4 is upregulated under anaerobic conditions, suggesting it is adapted for use in such environments. Here, we investigate the hypothesis that each pyocin evolved specifically for use in a different environment. Pyocin-susceptible strains of PAO1 were engineered and tagged with fluorescent markers, allowing them to be distinguished from wild-type during competitions. Colony competitions were performed to assess pyocin effectiveness under anaerobic, aerobic, low-iron, and high-iron conditions. Results indicated that S4, consistent with its upregulation, exhibited enhanced performance under anaerobic conditions. S2 provided a greater competitive advantage in high-iron conditions. Competition assays using pyocin-susceptible strains were performed, and revealed that S2 did not induce S5 expression, despite it being a DNase, and as a pyocin, it is induced by DNA damage. This suggests pyocin S5 may be regulated by other signals, including iron availability, which was supported by S5 performing better when iron was restricted. This possibility is also supported by analysis of the promoter regions of each pyocin, revealing distinct sequences. Observations of differential effectiveness between pyocins may therefore result from variations in both pyocin and receptor expression, supporting the hypothesis that each pyocin evolved for use under specific conditions, and explains why *P. aeruginosa* has many seemingly similar weapons.

Gen abstract type

Poster

Section Type

MGCM

59 - Defining the factors needed for rapid colony expansion during exploratory growth in *Streptomyces venezuelae*

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The bacterial cell wall is an essential protective barrier and is one of our best antibiotic targets. Bacteria have evolved distinct strategies to build and remodel their cell wall during growth. Some grow using lateral (side) wall synthesis, which is directed by protein MreB, while others employ polar growth, which in *Streptomyces* and their relatives, is directed by the protein DivIVA. DivIVA localizes to the cell pole, where it recruits other proteins to form a complex known as the 'polarisome', that directs the cell wall biosynthetic machinery. Two key polarisome members are the cytoskeletal proteins Scy and FilP. DivIVA is also post-translationally regulated by phosphorylation, through the competing activity of the kinase AfsK and the phosphatase SppA. Phosphorylation of DivIVA results in its release from the cell pole, allowing it to form new branch sites. Historically, bacteria were thought to grow exclusively by either polar or lateral cell wall synthesis. However, work from the Elliot lab has revealed that both strategies are required during *Streptomyces* "exploratory growth", a recently discovered developmental mode that is characterized by rapid colony expansion across solid surfaces. The discovery that dual cell wall biosynthetic strategies are essential for the viability of exploring cultures fundamentally changes how we view bacterial cell wall synthesis. In addition to this unique growth strategy, we have also discovered that DivIVA is subject to novel modifications during exploration in the form of C-terminal cleavage. To test how DivIVA cleavage impacts exploration, we created truncated and full-length DivIVA overexpression constructs and probed how these impacted the exploratory growth of *Streptomyces venezuelae*. Both overexpression strains exhibited enhanced growth compared with the wild-type parent strain, although the truncated variant displayed the fastest initial growth. These results suggest that DivIVA truncation promotes more efficient exploration. To determine the importance of DivIVA phosphorylation to exploration, we deleted both *afsK* (kinase) and *sppA* (phosphatase) and tested their ability to explore. The Δ*afsK* mutant was defective in its exploration capabilities compared with the wild-type, while the Δ*sppA* mutant exhibited enhanced exploration. We also deleted the polarisome-encoding members *scy* and *filP*. We found the Δ*scy* mutant did not explore as robustly as wild-type, while the Δ*filP* mutant exhibited enhanced exploration. These results suggest that DivIVA-interacting members are important for exploration. We next tested whether altering the polarisome composition, or modulating the phosphorylation status of DivIVA impacted its cleavage. No significant changes were observed in the Δ*filP* mutant background, but the Δ*scy* mutant showed decreased cleavage during later exploration, suggesting that Scy may contribute to recruiting the protease responsible for DivIVA cleavage. Additionally, DivIVA cleavage was increased in the Δ*sppA* mutant and decreased in the Δ*afsK* mutant, particularly during early exploration. These results support a model in which DivIVA phosphorylation promotes DivIVA cleavage during the early exploration stages. This work provides new insights into a novel bacterial cell wall synthesis strategy, including unveiling a unique modification of an essential cell wall biosynthetic factor. Overall, our findings are revealing unexpected plasticity in the synthesis of this outstanding drug target.

Gen abstract type

Poster

Section Type

MGCM

61 - The impact of evolution under repeated bottlenecks across different fluconazole concentrations on competitive fitness and genomic variation in *Candida albicans*

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Candida albicans is the most prevalent human fungal pathogen and is characterized by extensive phenotypic flexibility and genome plasticity, enabling its survival across diverse host-associated and environmental conditions. *C. albicans* is a fungal microbe that is a common commensal member of the healthy human microbiota. It is also an opportunistic pathogen with a knack for developing drug resistance. Previous works have shown that environmental stresses, particularly antifungal drug exposure, can induce aneuploidy and loss of heterozygosity in *C. albicans*. However, it remains challenging to decide whether a specific genomic change is neutral or beneficial, even when it is repeatedly observed, as stress may both cause and/or select for similar mutations. In this study, I will explore how population size and drug concentration influence the dynamics of drift and selection in *C. albicans*. To do this, I compared the distribution of mutations that arose when replicate lines evolved at low population size in the presence and absence of different levels of the putatively mutagenic drug fluconazole. Then, the evolved replicates were phenotyped to determine whether the mutations were deleterious, neutral, or beneficial. To facilitate this, I conducted the first mutation accumulation experiments done with *C. albicans*. Replicates evolved in the rich medium YPD and in fluconazole at different drug concentrations (0, 0.25, 1, 4, 8, and 16 µg/ mL). In each environment, between 12 ancestral lines were repeatedly bottlenecked to a single cell for 10 transfers. This experimental framework is typically used so that drift, rather than selection, is the primary evolutionary force. The competitive fitness of evolved lines in different fluconazole concentrations was quantified against a fluorescently labelled ancestor using a novel high-throughput method I developed during my M.Sc. To investigate the genomic changes, whole genome sequencing was performed on a subset of evolved lines, followed by variant identification and functional analysis, including gene ontology enrichment. Both the concentration at which *C. albicans* lines evolved and the concentration at which they were assayed influenced the competitive fitness. Lines evolved under higher fluconazole concentrations consistently exhibited greater competitive fitness compared to those evolved under lower concentrations. Genomic analysis showed that most detected variants were intergenic or synonymous, consistent with patterns expected under neutral mutation accumulation. In contrast, chromosomal aneuploidy was frequently observed in fluconazole-evolved lines, particularly involving chromosome R, and was absent in lines evolved without drug exposure and lines evolved under the lowest drug concentration (0.25 µg/ mL fluconazole). The frequency of aneuploidy increased with drug concentration, although its direct contribution to the increased fitness remains unresolved. Gene ontology analysis revealed genes were associated with hyphal growth in populations evolved under the highest drug concentration (16 µg/ mL fluconazole), and ongoing work is evaluating corresponding phenotypic changes. Overall, these results demonstrate that fluconazole concentration plays a critical role in shaping evolutionary outcomes in *C. albicans*, promoting rapid adaptation and increased competitive fitness even under repeated bottlenecks. This study highlights the importance of integrating phenotypic and genomic approaches to better understand the mechanisms underlying evolution to antifungal drugs.

Gen abstract type Poster

Section Type MGCM

63 - Rapid Point-of-Care Detection of Fluconazole-Resistant *Candida* from Oral Candidiasis

Anu Nair[1, 2], Chibike Ijomah[2], Michael Reich[2, 3], Yamamah Al-Jumaili[2], Dr. Karolina Czajka[3, 4], Jessica Arcand[3], Dr. Stacey Santi[1], Dr. Krishnan Venkataraman[2], Dr. Chris Verschoor[1, 3], Dr. Ravi Singh[5], Dr. Vasu D. Appanna[2], Dr. Deborah P. Saunders[5], Dr. Danielle Brabant-Kirwan[5], Dr. Sujeenthar Tharmalingam[3]

[1]Health Sciences North Research Institute, [2]Laurentian University, [3]Northern Ontario School of Medicine (NOSM), [4]Laurentian Univeristy, [5]Health Sciences North

Background Oral candidiasis, a prevalent opportunistic infection caused by Candida species, poses significant challenges due to rising fluconazole resistance in pathogens like *C.glabrata*, *C. krusei*, and *C. auris*. Traditional diagnostics relying on culture and molecular methods are slow, delaying targeted therapy and exacerbating healthcare burdens. This study introduces a rapid point-of-care platform combining Loop-Mediated Isothermal Amplification (LAMP) with CRISPR-Cas12 detection to identify resistance-associated loci in four key species: *C. albicans*, *C. glabrata (Nakaseomyces glabrata)*, *C. krusei* (*Pichia kudriavzevii*), and *C. auris* (*Candidozyma auris)*.

Methods Genomic sequences were mined for species-specific markers genes. In silico design yielded 4-6 primer sets (including loops) optimized for Bst polymerase activity at 60-65°C. DNA from lab cultures underwent serial dilution for limit-of-detection (LOD) testing. LAMP reactions incorporated dNTPs, Mg^{2+}, and fluorescent/colorimetric dyes, monitored over 60 minutes. CRISPR-Cas12 integration using guide RNAs for collateral cleavage of reporters, with temperature optimization for one-pot compatibility. Assay robustness is assessed via fluorescence intensity, signal timing, and reproducibility across species, simulating clinical conditions.

Results LAMP achieved amplification in 30-60 minutes, with robust signals above 10^4 cells/reaction across species. Sensitivity waned below 500 cells/reaction, revealing interspecies variability in timing and intensity. CRISPR enhancement sharpened signals, boosting LOD and discrimination, especially for low-abundance targets. Optimized conditions supported dual-enzyme function, paving the way for streamlined workflows.

Discussion While LAMP offers isothermal speed, genetic diversity demands refined targets for consistency. CRISPR addresses this by amplifying specificity, yielding a superior hybrid for resource-limited settings. Clinically, this could accelerate stewardship, curbing misuse of fluconazole amid rising resistance.

Conclusion The LAMP-CRISPR platform shows promise for 60-minute detection of resistant *Candida*, with CRISPR mitigating LAMP's variability. Next steps include clinical validation, target refinement, and one-pot formatting for bedside use, potentially revolutionizing fungal diagnostics.

Gen abstract type

Poster

Section Type

MGCM

67 - Determining the mechanism of Ku-LigD mediated DNA double strand break repair in *Mycobacterium tuberculosis*

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DNA damage in bacteria often arises from a combination of environmental stressors or metabolic by-products. The most lethal form of DNA damage is a double-strand break (DSB), and in many prokaryotes, these are repaired through non-homologous end-joining (NHEJ), an error-prone process as it does not require a DNA template. NHEJ predominates during non-dividing states in prokaryotes, such as stationary phase or a latent infection; the latter of which poses challenges for bacterial eradication. Introducing DNA DSBs is a common bactericidal mechanism adopted by quinolone antibiotics and the immune system. The pathogen *Mycobacterium tuberculosis,* which is the causative agent of tuberculosis, killing 1.5 million people every year, is tolerant to this strategy due to a robust NHEJ system. Consequently, when damaged DNA is repaired the bacterium can accumulate chromosomal mutations which can confer antimicrobial resistance The critical proteins involved in prokaryotic NHEJ are Ku and LigD. Prokaryotic Ku includes a core domain which binds to the DSB, and a unique C-terminal tail that is responsible for recruitment of the multifunctional ligase, LigD. LigD carries out ligase, phosphoesterase/nuclease, and polymerase activities, each facilitated by a distinct domain. Ku is known to influence the activity of both the ligase and polymerase domains of LigD through direct interactions, however, the effects of Ku on the phosphoesterase domain are still unknown.

Using established assays, I showed that purified *M. tuberculosis* phosphoesterase domain alone was biologically inactive and incapable of binding a DNA substrate *in vitro*, unlike the other LigD domains. Follow-up assays conducted using phosphoesterase domain truncations that included one or both of the other LigD domains revealed that phosphoesterase function is stimulated by either the polymerase or the ligase domain. This suggested that phosphoesterase function may require allosteric input from the additional domain(s) to promote the active conformation, or to allow for DNA binding. Further addition of Ku to WT LigD, and the truncations containing the other domains revealed that Ku attenuates phosphoesterase activity. The activity of the polymerase-phosphoesterase domain construct was significantly impacted, which indicates that the Ku contact through the polymerase domain could be allosterically communicated to inhibit phosphoesterase activity. To probe whether Ku inhibits activity through a direct interaction with the phosphoesterase domain, microscale thermophoresis was performed. The phosphoesterase domain interacted weakly with Ku, with a K_d in the micromolar range. Ku has nanomolar affinity for both the polymerase and ligase domains of LigD, and this result suggested that a direct Ku-phosphoesterase domain interaction was not biologically significant. To parse out whether Ku regulates the phosphoesterase domain through allostery, structural work has been ongoing. To perform cryo electron microscopy, we have formed a stable ternary complex of Ku-LigD and DNA which has been verified through dynamic light scattering and electromobility shift assays. Complex integrity has been validated under cryogenic conditions, and promising grids are currently awaiting data collection. Taken together, these data will delineate critical functional interactions required for species-specific regulation of LigD by Ku and potentially could inform on sites for downstream design of new therapeutics.

Gen abstract type Poster

Section Type MGCM

71 - Investigating the impact of D-amino acids on β-lactam resistance and outer membrane integrity

Montserrat Mora Ochomogo[1], Kristen Mawdsley[1], Seojin (Jinny) Yu[1], Christopher T. Lohans[1]

[1]Queen's University

β-lactam antibiotics are among the most widely used antimicrobials, yet their efficacy is increasingly compromised by β-lactamase-producing bacteria. Beyond conferring resistance, β-lactamases can promote antibiotic "sheltering," protecting nearby susceptible bacteria and contributing to treatment failure, particularly in polymicrobial infections. Because β-lactamases are located in the periplasm of Gram-negative bacteria and peptidoglycan composition influences cell envelope integrity, we investigated how incorporation of specific D-amino acids into peptidoglycan alters β-lactamase release and antibiotic hydrolysis in *Escherichia coli* and *Staphylococcus aureus*.

KPC-2-producing *E. coli* and ATCC strains of MSSA and MRSA were cultured in the presence of various D- and L-amino acids, and β-lactamase activity was measured using nitrocefin hydrolysis assays. Among the amino acids tested, D-tryptophan (D-Trp) showed the highest nitrocefin hydrolysis velocity, resulting in a four-fold increase in nitrocefin hydrolysis in *E. coli* compared to L-Trp-treated and untreated controls. D-Trp also increased β-lactamase activity in both *S. aureus* strains, though to a lesser extent. Higher hydrolysis was similarly observed in *E. coli* expressing additional β-lactamases (OXA-48 and IMP-1) and was dose dependent, with the highest effects at higher D-Trp concentrations.

Testing cells and culture supernatant showed that increased hydrolytic activity following D-Trp treatment was primarily in the culture supernatant. Consistent with this, Western blot analysis of a FLAG-tagged KPC-2 strain demonstrated elevated levels of β-lactamase in the supernatant after D-Trp exposure, indicating increased enzyme release rather than enhanced catalytic activity alone. Transmission electron microscopy did not reveal membrane damage, and nitrocefin hydrolysis tests carried out in outer membrane vesicles did not show a difference when comparing D-Trp and L-Trp. Notably, the D-Trp effect of increased nitrocefin hydrolysis was not shown in an *E. coli* Δ*nlpI* mutant while in other *E. coli* knockouts tested (Δ*lpp* and Δ*ompA*) D-Trp showed a higher rate of nitrocefin hydrolysis, suggesting NlpI has at least a partial role in this process.

Together, these findings demonstrate that specific D-amino acids can promote β-lactamase release by destabilizing cell wall interactions, enhancing antibiotic hydrolysis and potentially contributing to antibiotic sheltering in polymicrobial environments.

Gen abstract type

Poster

Section Type

MGCM

76 - Northern Ontario Candida Isolates Showcase Strain-Specific Transcriptional Responses Play a Role in Azole Resistance

Yamamah Al-Jumaili[1, 2], Karolina Czajka[2], Anu Nair[1], Michael Reich[1], Jessica Arcand[1], Krishnan Venkataraman[1], Danielle Brabant-Kirwan[3], Stacey Santi[4], Chris Verschoor[2, 4], Vasu Appanna[1], Ravi Singh[3], Deborah P. Saunders[2, 4], Sujeenthar Tharmalingam[2]

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Oral candidiasis is one of the most common fungal infections in the oral cavity of humans. It is caused by the overgrowth of naturally occurring *Candida* fungal species in the oropharynx and debilitates a wide range of the population, primarily the immunocompromised and elderly. An emerging issue in treatment is species resistance to azole antifungals. This study characterized antifungal resistance mechanisms in twelve Northern Ontario clinical isolates, including *C. albicans* (n = 3), *C. glabrata* (n = 5), and *C. tropicalis* (n = 5) strains, later classified as susceptible, intermediate, or resistant based on growth patterns. Strains were exposed to fluconazole and clotrimazole, and expression of target genes were assessed relative to the susceptible control. Significant strain and species-specific responses were observed following azole exposure. *ERG11* was significantly upregulated in fluconazole-treated *C. albicans* susceptible (4.3 fold increase) and intermediate strains (5.8 fold increase) ($p < 0.05$), and in the clotrimazole-treated resistant strain (6.9 fold increase) ($p < 0.001$). All *C. tropicalis* strains similarly demonstrated increased *ERG11* expression following azole treatment. Notably, the resistant *C. albicans* isolate was found to have a methionine-to-isoleucine substitution at position 651 (M651I) in *UPC2*, suggesting altered regulatory activity. Additionally, *HWP1* expression showed a significant 3.19-fold increase in the susceptible strain compared to the resistant strain at baseline. Resistant isolates demonstrated higher expression of genes related to efflux pumps as well as stress, supporting the multifaceted nature of antifungal resistance. These findings identify *ERG11* upregulation, and *UPC2* and *HWP1* variation as key contributors to azole resistance in Northern Ontario clinical isolates. Ongoing validation studies using CRISPR-mediated gene knockout and plasmid-based overexpression systems aim to confirm the functional contribution of these genes to resistance phenotypes, and strengthen our understanding of the molecular mechanisms underlying antifungal resistance in Northern Ontario *Candida* isolates.

Gen abstract type

Poster

Section Type

MGCM

78 - YfgH is a transmembrane glycine-zipper containing lipoprotein that stabilizes excess cardiolipin and outer membrane proteins during envelope stress

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The outer membrane (OM) in Gram-negative bacteria is an asymmetric phospholipid (PL) - lipopolysaccharide (LPS) bilayer. This membrane is essential for survival of Gram-negative bacteria such as Escherichia coli (E. coli); therefore, its composition and asymmetric nature is tightly regulated. Many different proteins/pathways have been characterized as working together to maintain this asymmetrical nature, such as the Mla pathway (which transports mislocalized PL to the inner membrane) and the phospholipase PldA (which degrades mislocalized PL). A recent study suggested that a transmembrane glycine zipper domain-containing lipoprotein, SlyB, could respond to the loss of asymmetry of the OM by oligomerizing into ring-like structures that stabilize encapsulated OM proteins in lipid nanodomains and break up phospholipid islands in the outer leaflet. Deletion of slyB leads to decreased growth of cells when exposed to OM stress, such as metal chelators and detergents, and a decrease in global OM protein profile. While it was hypothesized that SlyB represents a more prominent family of glycine zipper-containing lipoproteins that stabilize the OM during stress, the identity and function of other members of this family remain unknown.

In this study, we report that the uncharacterized lipoprotein YfgH is a structurally and phenotypically similar but distinct OM-stabilizing protein to SlyB. YfgH is an envelope stress response regulated lipoprotein that contains a transmembrane glycine-zipper domain. Deletion of yfgH sensitized cells to OM stress, such as metal chelators and detergents, and increased OM permeability. Further, YfgH is necessary for survival when OM composition and asymmetry is disrupted through CRISPR interference (CRISPRi) of OM biogenesis genes; YfgH overexpression partially or completely rescues cell growth during CRISPRi knockdowns of these genes. We used AlphaFold3 to predict potential higher-order structures for YfgH because transmembrane glycine-zipper domains are often found to form oligomeric structures. The resulting model predicted that YfgH would form ring-like oligomeric structures, similar to those formed by SlyB when stabilizing a compromised OM. To determine if these oligomeric structures of YfgH and SlyB stabilize the OM in similar or distinct ways, we isolated suppressors to the OM destabilizing phenotypes of yfgH and slyB deletions. Suppressors of yfgH were found to be deactivating mutations of the major cardiolipin synthase clsA, whereas slyB suppressors were found to be mutations that disrupt the Mla pathway.

This model ultimately suggests that YfgH is stabilizing the OM by a mechanism distinct from SlyB's response to the loss of OM asymmetry. Our results provide structural and genetic insight into an emerging family of glycine zipper-containing lipoproteins that stabilize the OM in distinct ways during stress.

Gen abstract type Poster

Section Type MGCM

84 - Triclosan rewires efflux regulation in *Acinetobacter baumannii*: AdeIJK induction independent of AdeN

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Exposure to non-antibiotic antimicrobial agents can promote cross-resistance to antibiotics in pathogenic bacteria. Nevertheless, the regulation of such resistance mechanisms is not fully understood. This study investigates the gene regulatory mechanisms by which the non-antibiotic antimicrobial triclosan contributes to multidrug resistance (MDR) phenotype in *Acinetobacter baumannii*, a notorious opportunistic pathogen responsible for bloodstream, wound, and lung infections in humans. *A. baumannii* is classified as a top-priority pathogen, as a significant proportion of isolates exhibit resistance even to last-resort antibiotics.

We performed an adaptive evolution experiment by exposing wild-type *A. baumannii* to stepwise, two-fold increasing concentrations of triclosan. This resulted in the isolation of strain AB043, which showed a 64-fold increase in triclosan resistance compared to wild-type. AB043 also displayed cross-resistance to several clinically used antibiotics (Eg: Tigecycline, Ciprofloxacin). Transcriptomic analysis of AB043 compared to wild-type revealed upregulation of the *adeIJK* operon, a predominant multidrug-resistant efflux pump in *Acinetobacter* spp. belonging to the Resistance-Nodulation-Division (RND) family. AdeIJK upregulation is often associated with MDR phenotype in *A. baumannii*, as the pump expels structurally unrelated substrate antibiotics. Using an *adeIJK* knockout and genomic complementation strains, we confirmed that triclosan is a poor substrate of the pump, consistent with our previously published findings.

Further investigation of the regulatory mechanism of *adeIJK* was largely informed by whole-genome sequencing. No mutations were detected in *adeN*, the TetR-family repressor that primarily regulates *adeIJK* expression. However, a single-nucleotide variation (SNV), C-to-T was identified 230 bp upstream of *adeI*. Relative cDNA quantification across the 100 bp region immediately upstream and downstream of this SNV showed a significant increase in expression, extending throughout the *adeIJK* operon. This *adeIJK* induction is far exceeding the derepression caused by AdeN inactivation. We fused this region to a reporter gene and compared activity in the presence and absence of AdeN, demonstrating that the SNV introduces an alternative effector binding site and drives upregulation independently of AdeN. Our bioinformatic analysis further supports that AB043 undergoes membrane, oxidative, and metabolic stress, triggering upregulation of genes associated with biofilm formation and toxin secretion, reflecting a coordinated global stress adaptation.

Efflux pumps are traditionally recognized for their role in antibiotic export, yet increasing evidence highlights their involvement in core physiological processes of bacteria. Our findings indicate that triclosan exposure may induce physiological stress that triggers upregulation of the AdeIJK efflux pump via an alternative regulatory mechanism. This underscores the complex regulation of efflux systems in response to non-antibiotic stressors and raises critical concerns for opportunistic pathogens such as *A. baumannii*, which inhabit diverse environmental niches and human-associated settings, where such exposures may promote the emergence of multidrug resistance.

Gen abstract type

Poster

Section Type

MGCM

90 - Cataloguing Antifungal Resistance Across Fungal Diversity

Suhayb Hafiz[1], Andrew McArthur[1], Cameron Currie[1], Brian Alcock[1]

[1]McMaster University

Fungal infections are responsible for 1.5 to 4.5 million deaths globally per year. With only four systemic drug classes available, any emerging resistance limits clinical treatment options. Understanding which organisms carry resistance is critical, yet the tools available to detect and interpret it were never built with fungal biology in mind.

To address this gap, we integrated FungAMR into the Comprehensive Antibiotic Resistance Database (CARD). FungAMR spans 95 species, 359 genes, and over 9,864 unique mutations compiled across over 500 published studies. Its integration warranted a fundamental restructuring of CARD's Antibiotic Resistance Ontology (ARO). CARD's existing architecture was built around bacterial logic, where resistance is primarily mediated by genes acquired through horizontal gene transfer. Unlike bacteria, fungal resistance is predominantly mutational and vertical where genes confer resistance through specific divergence from a susceptible ancestral state. Two new principles were established in CARD to encode this distinction. First, every antifungal resistance gene must link to a term describing its biological function, grounding resistance in mechanism rather than treating it as an isolated annotation. Second, every mutated resistance gene must contain a branch back to a wild-type, antifungal-susceptible reference term within the ontology. This design distinguishes genes where specific point mutations confer resistance, from regulatory genes where overexpression is sufficient.

Beyond these two structural principles, additional categories were introduced for transcription factors, recognizing their mechanistically distinct role in mediating resistance through downstream transcriptional regulation. Copy number variation was also encoded as an additional resistance mechanism, capturing gene dosage as a route to phenotypic change. Furthermore, the cytochrome P450 family, consisting of ERG4, ERG11, and related ergosterol biosynthesis genes, was placed under a shared hierarchical parent, enabling family-level queries and reflecting their common biochemical ancestry. Taken together, these efforts address a gap that existed before this restructuring, where no pathway existed within CARD to traverse from a resistance phenotype to its implicated mechanism, gene, and original susceptible sequence for a fungal organism. The result is over 400 new ontology terms across 57 fungal species housing 109 resistance genes, enabling phylogenetically structured queries of antifungal resistance within a standardized framework.

With CARD's curated knowledge base now including several species across the fungal tree of life, the database enables comparative analyses of resistance distributions previously unsupported by any existing tool. This will determine which resistance mechanisms are conserved across distant clades, and which arise convergently in unrelated lineages. This distinction could directly inform how antifungal resistance evolves and spreads across the fungal kingdom. To extend this analysis into poorly annotated or understudied lineages, we also propose a dual-component architecture consisting of a Hidden Markov Model (HMM) to capture conserved sequence-level profiles of resistance genes, and a Protein Language Model (PLM) trained on the mutational signatures encoded in the ontology to distinguish resistant variants from their susceptible wild-type sequences. In understudied clades, sequence divergence from characterized orthologs renders homology-based detection unreliable. Together, these tools aim to probe resistance in regions of the fungal kingdom where genomic data exists but biological interpretation does not.

Gen abstract type Poster

Section Type MGCM

91 - Broad-Scale Identification, Classification, and Characterization of Prophages in 615 *Pantoea* Genomes

Mohammed Quraishi[1], John Stavrinides[1]

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Bacteriophages facilitate the movement of novel genetic material between bacteria, impacting the virulence, metabolism and ecological fitness of their hosts and helping to drive bacterial evolution. Members of the genus *Pantoea* occupy a wide variety of niches as saprophytes, plant pathogens and insect mutualists, and even as opportunistic human pathogens. Very little is known about the diversity of prophages that may have contributed to genetic novelty and the diversification of this group. We conducted a large-scale survey of prophages across 615 *Pantoea* genomes, representing 43 species. Prophage were identified using PHASTEST, yielding 1076 prophages across 510 genomes. Most strains of *Pantoea* harbor between zero and five intact prophages; however, some strains carry up to 11 prophages. Prophage range from 6.1 kb to 103.5 kb in size, and encode between 9 and 152 predicted open reading frames. VContact3, VClust and vOTU clustering classified 1076 prophages as belonging to 333 viral genera across 36 viral families. Most of these groupings are unknown, with 330 genera and 3 families being novel, and have yet to be fully characterized. The most abundant phage family was Peduoviridae, which exhibited a broad host range across *Pantoea* species, followed by multiple novel lineages of *Caudoviricetes*. Prophages are not evenly distributed, with species such as *P. agglomerans* and *P. ananatis*, known for their ecological versatility, harboring the greatest abundance and diversity of phages. A sequence similarity-based search of these phages against the NCBI nucleotide database suggests that they are restricted to members of *Pantoea*, except for one prophage that was identical to a prophage identified in *Acinetobacter*. These results indicate that *Pantoea* genomes contain a large and highly diverse set of undescribed and uncharacterized prophages. The observed diversity of prophages mirrors the ecological and taxonomic diversity of their bacterial hosts, suggesting that bacteriophages have contributed to the diversification of the genus. This study provides the first comprehensive overview of prophage diversity in *Pantoea* and establishes a foundation for future investigation into the functional roles of prophages in shaping bacterial evolution, adaptation, and pathogenic potential.

Gen abstract type

Poster

Section Type

MGCM

93 - Iron acquisition pathways in the emerging multidrug-resistant and opportunistic pathogen *Morganella morganii*

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Morganella morganii is an emerging multidrug-resistant (MDR) bacterium that functions primarily as a commensal on mucosal surfaces but readily becomes opportunistic under conditions of host stress. *M. morganii* is increasingly viewed through a One Health lens because it circulates among humans, animals, food systems, and environmental reservoirs while readily acquiring and disseminating antimicrobial-resistance determinants. Despite its broad distribution and growing clinical relevance, vanishingly little is known about the basic biology of this organism, including the molecular systems it relies on to acquire essential nutrients. Iron is one such nutrient. It is required by nearly all organisms and plays a central role in numerous cellular processes. Because of this, disruptions in iron homeostasis can profoundly affect bacterial fitness. In many environments, particularly under physiological conditions within the host, iron is both highly insoluble and sequestered as a facet of nutritional immunity. As such, iron is scarcely available to most organisms. This scarcity has driven bacteria to evolve specialized strategies to scavenge iron from their surroundings. Some bacterial species directly utilize host iron-containing proteins such as transferrin, lactoferrin, or hemoglobin, while others are able to take up heme as an iron source. Many microorganisms also synthesize and secrete siderophores, which are small, high-affinity iron-binding molecules that efficiently chelate ferric iron. Sequencing performed by our lab on modern clinical isolates of *M. morganii* reveals no detectable loci for siderophore synthesis. This observation raises important questions about how *M. morganii* acquires iron in the absence of endogenous siderophore production. This project therefore aims to identify the key iron uptake pathways and preferred source(s) of metal for *M. morganii*. RNA sequencing of cells exposed to iron-deplete and iron-replete conditions revealed genes that were highly upregulated under iron limitation. These genes are predicted to encode systems involved in the uptake of ferric citrate, ferrous iron, and exogenous (xeno)siderophores produced by other organisms, as *M. morganii* does not synthesise its own siderophores, suggesting that they form part of the bacterium's iron-acquisition machinery. This analysis also revealed additional iron transport-related clusters that may enhance our understanding of how *M. morganii* maintains iron homeostasis. Ongoing growth curve analyses are being conducted to evaluate how different iron sources contributed to iron-dependent growth under nutrient-limited conditions. Our data to date indicate that *M. morganii* is capable of using ferric complexes, host iron sources, as well as xenosiderophores as iron sources, and that the metal is indeed required for its growth. Insights from this study may therefore inform future therapeutic development for MDR *M. morganii*. In future work, we aim to investigate these pathways further by generating mutants in key genes and assessing their roles in iron-dependent growth.

Gen abstract type

Poster

Section Type

MGCM

94 - Determining the impact of iron excess on the physiology of *Morganella morganii*, an emerging superbug

Tanasha Iftekhar[1], Christine Joyce Francisco[1], Enrique Aburto Arreguin[1], Camille Hamula[2], Ninad Mehta[2], Jessica Sheldon[1]

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Morganella morganii is a commensal of humans and animals, that is increasingly garnering attention as an emerging multidrug-resistant, opportunistic pathogen. It is a causative agent of urinary tract infections, wound infections, meningitis, and sepsis. As a member of the SPICE (*Serratia*, *Providencia*, indole-positive *Proteus*, *Citrobacter*, and *Enterobacter* species) group of inducible β-lactamase producing Enterobacter, *M. morganii* exhibits intrinsic and inducible resistance mechanisms that complicate treatment and promote therapeutic failure. Despite its growing clinical significance, the molecular basis of its stress adaptation, environmental persistence, and virulence remains poorly defined. Iron availability is a key factor of bacterial pathogenesis, functioning both as an essential micronutrient and as a potential intoxicant when present in excess. While iron starvation responses have been extensively characterized in other organisms, adaptive mechanisms that mitigate iron intoxication remain comparatively underexplored. In this study, we therefore investigated the transcriptional and phenotypic responses of *M. morganii* wild-type strain JRS 2-97 to high iron conditions (100 μM $FeCl_3$), a concentration that reproducibly induces formation of a distinct black extracellular compound. Exposure to excess iron resulted in robust and reproducible production of a glucose repressible black cryptic compound and triggered a defined transcriptional response. RNA sequencing identified 13 genes with statistically significant differential expression under iron-rich conditions (adjusted $p \leq 0.05$). Among these, multiple highly upregulated loci ($\log_2$fold change ≥ 1) encoded for putative efflux transporters, including components of the MdtABC multidrug efflux system, regulatory elements, and several proteins containing domains of unknown function (e.g., DUF305, DUF4822). These transcriptional changes suggest a coordinated response by metal detoxification, oxidative stress mitigation, and/or export associated pathways in response to excess iron, rather than a generalized stress response. Furthermore, we observed that the production of the black compound is strain-dependent and absent in a subset of clinical isolates, suggesting phenotypic variability that may reflect niche adaptation, and laying the groundwork for comparative genomics to identify potential contributing loci. Given excess iron can promote metalloprotein mismetallation and reactive oxygen species formation, this compound may represent a previously undescribed mechanism of iron sequestration, or detoxification. We are currently employing transcriptomics, mass spectrometry, targeted mutagenesis, and comparative genomics to define the chemical identity and functional contribution of the black cryptic compound to iron homeostasis. Our results indicate iron intoxication represents a previously underappreciated stress in *M. morganii* and provides insight to pathways involved in iron homeostasis. Elucidating these mechanisms advances understanding of the basic biology of this emerging pathogen and may help identify novel targets for antimicrobial intervention.

Gen abstract type Poster **Section Type** MGCM

95 - Genome-Guided Identification of Novel Antimycobacterial ClpP Modulators

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Protein homeostasis is a fundamental requirement for cellular viability, relying on the precise balance between protein synthesis and degradation. In bacteria, the ATP-dependent ClpXP protease complex plays a critical role in maintaining this balance by selectively degrading misfolded, damaged, and regulatory proteins. In pathogenic organisms such as *Mycobacterium tuberculosis*, ClpXP is essential for survival, stress adaptation, and virulence, making it an attractive yet underexplored target for antibiotic development. Despite its importance, few small-molecule modulators of ClpXP have been identified, with acyldepsipeptides (ADEPs) representing one of the only well-characterized natural product classes capable of dysregulating its activity.

Microbial natural products remain a rich source of structurally diverse bioactive compounds; however, traditional discovery approaches are often hindered by the rediscovery of known compounds. Genome-guided strategies that leverage self-resistance mechanisms encoded within BGCs have emerged as powerful tools for identifying novel bioactive molecules. Here, we apply a genome-guided approach targeting *clpP*-associated BGCs to identify new natural product modulators of the ClpXP protease.

Using a custom bioinformatic pipeline, we screened genomes from the Wright Actinomycete Collection (WAC), a unique collection of >12,000 natural product-producing bacteria and fungi isolated over the past 25 years This analysis identified approximately 70 clpP-associated BGCs across 65 strains. These strains were then fermented and their crude extracts were screened using a cell-based reporter system in *Streptomyces coelicolor*, in which β-glucuronidase expression is driven by the clpP3 promoter. Activation of this reporter indicates disruption or dysregulation of ClpP1P2 activity, resulting in a visible blue phenotype.

Screening results revealed that 11 strains reproducibly produced metabolites capable of modulating ClpP activity. These active strains corresponded to diverse BGC families, including hybrid polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) systems, Pls/PosA-like NRPS clusters, and phosphonopeptide-associated pathways. Subsequent biochemical analysis using purified *M. tuberculosis* ClpP confirmed that these metabolites directly influence ClpP activity, with the majority functioning as inhibitors and one strain exhibiting activation consistent with ADEP-like dysregulation.

Two representative strains, WAC02706 and WAC07405, were selected for further investigation. WAC02706 produces a putative ClpP activator and contains two distinct clpP homologs within a BGC related to alpiniamide biosynthesis, suggesting a potential self-resistance mechanism. In contrast, WAC07405 encodes a complex BGC with features resembling antipain and phosphonate pathways and produces a ClpP inhibitor with a potentially novel mechanism of action. Ongoing activity-guided purification and structural elucidation will help further define the nature of these compounds.

Collectively, this work demonstrates that targeting clpP-associated BGCs is an effective strategy for discovering novel modulators of the ClpXP protease. The identification of both inhibitors and activators highlights the diversity of ClpP-targeting natural products and underscores their therapeutic potential. This genome-guided framework provides a scalable approach for natural product discovery and expands the resevoir of strategies for developing new antimicrobial agents targeting essential proteostasis pathways.

Gen abstract type

Poster

Section Type

MGCM

97 - Development of an enzymatic mycobacterial lysis method for next-generation diagnostics

Cora England[1], Patrick Moynihan[1]

[1]Western University

Mycobacterium tuberculosis is the causative agent of tuberculosis, a respiratory pathogen which has led to more fatalities than any other infectious disease in human history. While early intervention is key to controlling tuberculosis mortality, its diagnosis is often heavily stigmatized and depends upon poor diagnostic tools, contributing to millions going undiagnosed and/or untreated each year. PCR-based testing, which is fast and accessible, could revolutionize tuberculosis diagnosis. However, the unique nature of the mycobacterial cell envelope presents a profound challenge in extracting the required template DNA from the bacteria. Traditional mycobacterial DNA extraction involves harsh and resource-intensive methods, and typically results in poor yields of low-quality DNA. We hypothesize that cell-wall degrading enzymes, which are used by mycobacteria throughout their life cycle to remodel their cell wall or by phage during cell lysis, could be employed as a more efficient and gentle approach to acquiring mycobacterial DNA. Unfortunately, due to the unique modifications observed in the mycobacterial cell wall, such as a conserved arabinogalactan layer and heavily modified peptidoglycan, canonically-used lysis enzymes such as lysozyme are ineffective against mycobacteria. While our laboratory recently discovered the first group of enzymes capable of completely depolymerizing arabinogalactan, we believe simultaneously targeting multiple cell wall structures to be essential for optimized lysis and consequently genomic DNA yield. To overcome the mechanical barrier of peptidoglycan, we sought to identify new lysins capable of depolymerizing this layer. We first screened available genomes of both mycobacteria and phages to determine the range of potential lytic enzymes. From this we identified nine mycobacterial and mycobacteriophage peptidoglycan-degrading enzymes, developed expression constructs, and purified the recombinant proteins. We then evaluated their enzymatic activity against native mycobacterial peptidoglycan using a fluorescence-release assay, prompting further characterization of these proteins. Data collected following an 18 h incubation period showed that four of these proteins were more active on mycobacterial peptidoglycan than the best available commercial enzyme, mutanolysin. Overall, developing a reliable enzymatic lysis method will enable not only the rapid diagnosis of tuberculosis and other mycobacterial diseases in patients, but will also provide antimicrobial resistance predication and support epidemiological contact tracing, all within a single clinical appointment.

Gen abstract type

Poster

Section Type

MGCM

107 - Evolutionary trajectory of GH38s in mycobacteria; A structure and function analysis

Suzanna Benn[1], Aaron Franklin[2], Patrick Moynihan[1]

[1]Western University, [2]University of Birmingham

Mycobacterium tuberculosis persists as a global health threat due to a lack of adequate diagnostics and vaccines. Lipoarabinomannan (LAM) is an antigenic cell envelope component unique to mycobacteria that is essential for cell envelope integrity and mediating host-pathogen interactions. LAM contains a mannan domain composed of an α-1,6 mannan backbone with α-1,2 mannose branches. Structural variations of this domain stimulate differing host immune responses, however, the enzymes involved in its remodelling are poorly understood. We have identified enzymes from the glycoside hydrolase 38 family (GH38s) as potential LAM remodelling enzymes due to their α-mannose specificity, cell envelope location, and conservation within the Mycobacteriales. Analysis of the sequence conservation of mycobacterial GH38s has revealed two groups of enzymes found in phylogenetically distinct groups of mycobacteria. These groups separate cleanly based on predicted quaternary structure. GH38s from *Mycobacterium abscessus* and closely related organisms are predicted tetrameric, which we have confirmed by solving a cryo-EM structure of the GH38 from *M. abscessus.* Preliminary characterisation of this enzyme confirms it has α-mannosidase activity. The second group are a novel sub-family which is predicted to be monomeric. Interestingly, we have identified that the *M. tuberculosis* type strain, H37Rv, has a severe truncation likely resulting in inactive protein. This protein is predicted to be functional in almost all clinical isolates, suggesting a domestication event. We have also shown that loss of this gene impacts the colony morphology and hydrophobicity of *M. bovis* BCG, suggesting a role in cell envelope biogenesis. This work fits into our broader aim of uncovering LAM remodelling mechanisms which will deepen our understanding of how *M. tuberculosis* alters its cell surface to manipulate the host immune system. These findings could also be exploited for increasing the specificity of existing *M. tuberculosis* diagnostics or vaccine development.

Gen abstract type

Poster

Section Type

MGCM

117 - Elucidating the Mechanistic Basis of *Escherichia coli* Efflux Pump-Mediated Acid Resistance

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[1]University of Guelph, Molecular and Cellular Biology

Survival of *Escherichia coli* during host colonization requires rapid adaptation to extreme and fluctuating pH environments, including passage through the highly acidic stomach, growth in the mildly acidic intestinal tract, and exposure to localized alkaline microenvironments. Drug efflux pumps are ubiquitous antimicrobial resistance (AMR) determinants that export antibiotics and other environmental stressors, thereby mediating resistance. In *E. coli*, the efflux network is highly conserved and predates the clinical use of antibiotics, suggesting broader roles in bacterial physiology. Indeed, efflux pumps have been implicated in multiple physiological processes, including pH homeostasis. However, extensive redundancy and overlapping substrate specificity among efflux pumps have historically obscured the contributions of individual systems.

To combat these challenges, an efflux-deficient strain (EKO-$35_{2.0WF}$) was developed to serve as a simplified background to study efflux pump functions. Individual efflux pumps are subsequently chromosomally integrated and constitutively expressed. Under pH stress, the fitness of EKO-$35_{2.0WF}$ is reduced, allowing us to explore the functions of individual efflux pumps. While the mechanistic basis of efflux-mediated alkaline tolerance is relatively well understood, their roles in acid resistance remain less defined. Growth profiling under alkaline stress (pH 9.5) identified a distinct set of single-component inner membrane pumps that restore fitness of EKO-$35_{2.0WF}$: MdtK, MdfA, YdhC, and EmrD. In contrast, multicomponent efflux systems play a critical role in acid resistance, highlighting the context-dependent nature of efflux-mediated fitness. At pH 4.25, five multicomponent efflux systems, AcrAB, AcrEF, MdtEF, EmrAB, and EmrKY, restore growth. Strikingly, under extreme acidity (pH 2), MdtEF alone restores survival of EKO-$35_{2.0WF}$, revealing a specialized and non-redundant role. These findings indicate that efflux systems transition from redundant contributors under moderate stress to essential determinants under extreme conditions.

To explore the mechanistic basis of acid resistance, we performed untargeted metabolomics using chemical isotope labelling LC-MS on WT_{WF}, EKO-$35_{2.0WF}$, and EKO-$35_{2.0WF}$::*mdtEF* across neutral, mildly acidic, and extremely acidic conditions. These data suggest that MdtEF is associated with coordinated shifts in polyamines and other stress-associated metabolites, consistent with a role in maintaining intracellular metabolic homeostasis during acid stress. Although several distinct metabolic pathways are significantly perturbed, the response is not confined to a single pathway and instead reflects widespread metabolic reprogramming.

Consistent with metabolomic changes in polyamine pathways, genetic perturbation of polyamine transport revealed that deletion of *potE* improves survival at pH 2 in both WT_{WF} and efflux-deficient backgrounds, while deletion of *cadB* enhances survival specifically in the efflux-deficient strain. These findings suggest that intracellular accumulation of putrescine is protective, likely through stabilization of macromolecules, whereas periplasmic cadaverine accumulation becomes detrimental in the absence of functional efflux.

Overall, these results identify MdtEF as a critical determinant of survival under extreme acid stress and support a model in which efflux pumps function as central regulators of intracellular metabolite homeostasis. More broadly, this work redefines multidrug efflux systems as key drivers of bacterial stress adaptation, extending their role far beyond antibiotic resistance.

Gen abstract type Poster

Section Type MGCM

125 - Polymorphism causes differential regulation of curli fimbriae in *Salmonella enterica* serovar Typhi

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Salmonella enterica can infect a broad range of hosts. While some serovars are generalists, others are host-restricted, such as serovar Typhi, which is human-specific. *S.* Typhi is endemic in many regions, and we are facing a major antibiotic resistance crisis. Chronic carriers harbour *S.* Typhi biofilm in their gallbladder, contributing to persistence and transmission. Curli-type fimbriae are key determinants of biofilm formation in *Salmonella*. Furthermore, anti-curli antibodies have been found in infected patients, suggesting their involvement during infection. Curli is encoded by two operons: *csgBAC* and *csgDEFG,* in which numerous polymorphisms (SNPs) have been identified in *S.* Typhi. Thus, we hypothesize that curli is distinctly regulated in *S*. Typhi. The objective of this study is to identify the regulators involved in curli expression.

Previously, we demonstrated that the main curli activator, CsgD, is unable to bind DNA due to a nonsense mutation. However, correction of this mutation did not restore the curli-positive phenotype, suggesting the existence of an alternative regulatory pathway. To identify alternative regulators, we performed a protein-DNA interaction capture approach targeting the curli promoter region and identified the EnvZ/OmpR two-component system. We demonstrated that EnvZ/OmpR exerts an inhibitory effect on curli expression, while it is an essential activator in other *Salmonella*. Mutants of this system exhibit increased biofilm biomass. The SNPs identified in the curli promoter region correspond to predicted OmpR binding sites. Furthermore, EnvZ itself carries mutations that reduce its activity. These SNPs potentially contribute to the strain-specific variations in curli expression observed in *S*. Typhi. Thus, understanding the distinct regulatory mechanisms of *S*. Typhi compared to other *Salmonella* will pave the way for the development of new antimicrobial treatments.

Gen abstract type

Poster

Section Type

MGCM

127 - Reversible biological motion with unidirectional catalysis through inversion of ATPase orientation

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Biological and engineered machines generally achieve reversibility through regulated switches in the directionality of fixed-orientation motors. Reversible biological motors are employed in the biosynthesis of bacterial type IV pili; dynamic bacterial surface appendages that undergo repeated cycles of extension and retraction. In the canonical type IVa pilus system, pilus polymerization and depolymerization is achieved through the dynamic exchange of dedicated extension and retraction motors composed of unidirectional hexameric ATPases and membrane-bound platform proteins. However, type IVc (Tad) pilus nanomachines extend and retract pili using a single motor with a unidirectional catalytic mechanism, a capability with no biological precedent.

Using AlphaFold3 modelling, comparative structural analyses, and pilus activity assays, we find that the *Caulobacter crescentus* Tad motor ATPase CpaF achieves bidirectionality through physical inversion; alternating which face of the ATPase toroid engages the platform complex. The two orientations contact the pilus machinery in mutually exclusive extension- or retraction-specific configurations that drive reversible pilus dynamics using the same unidirectional catalytic cycle. Retraction requires conserved C-terminal residues in CpaF whose nucleotide-driven motions oppose those of the extension interface.

Analysis of diverse extension- and retraction-specific Tad motor models uncovered platform protein conformational changes compatible with a model of steric control over the ATPase orientation permissible for pilus machine engagement. We propose that these conformations are regulated by the Tad-specific ParA/MinD-family ATPase CpaE. *In situ*, CpaE encircles the motor and undergoes large positional shifts between extension- and retraction-proficient pilus machines. These observations, combined with *in vitro* interaction data and modelling results hinting at contacts between CpaE and the conformationally-flexible regions of the platform proteins, position CpaE as the regulatory module of ATPase inversion. Extending this analysis to motors of the evolutionarily-related type IV filament superfamily suggests that ATPase inversion is a unique reversibility solution restricted to the bacterial Tad pili and their archaeal ancestor, the Epd pilus. Collectively, these results establish the Tad pilus as the prototypical example of a previously unrecognized mechanism of reversible biological motion.

Gen abstract type Poster

Section Type MGCM

128 - From Bench to Bedside in Northern Ontario: Expanding Clinical Validation of a Rapid LAMP-Based Assay for Fluconazole Resistance in Candida

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Candidiasis, the overgrowth of pathogenic Candida species, is a prevalent type of opportunistic fungal infection posing major health risks, especially for immunosuppressed, elderly, and palliative patients. The risk of mortality for these individuals increases if the infection is not treated in sufficient time. We hypothesize that early detection of fluconazole-resistance will help improve patient outcomes and reduce the risk of mortality and development of invasive fungal diseases (IFDs). The primary goal of this research proposal is to test the developed antifungal diagnostic assay on patient samples collected from Northern Ontario hospitals to assess its reproducibility by analyzing sensitivity across different strains and specificity among different Candida species. A previous clinical study was conducted, where 79 glycerol stocks were created from fungal swab samples retrieved from a cohort of 57 patients aged 65 or older-majority (65%) of whom were actively receiving cancer treatment-by the Health Sciences North (HSN) microbiology lab. The developed loop-mediated isothermal amplification (LAMP)-based assay demonstrated a limit of detection of 500 cells/µL, and achieved 100% sensitivity and 100% specificity for identifying fluconazole-resistant strains within a tested isolate set, in a prior study. The reproducibility of the assay will be tested with clinically relevant *Candida species*, including, *C. albicans, C. glabrata, C. auris, C. tropicalis, C. parapsilosis* and *C. krusei*, which have demonstrated an estimated resistance of 5-10% to azole drug fluconazole. Certain species like *C. auris* indicated higher rates of 87-100% resistance to fluconazole as reported by Public Health Ontario. This work directly addresses the emergence of antifungal resistance, particularly to the first line of Fluconazole treatment and supports the continued optimization of molecular diagnostic platforms for the detection of fluconazole resistance.

Gen abstract type

Poster

Section Type

MGCM

134 - Mitochondrial Impacts on Pleotropic Drug Resistance

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Pleiotropic drug resistance (PDR) in fungi is generated by a system of regulators and efflux pumps that can activate resistance to multiple, structurally unrelated xenobiotics following exposure to a single compound. Key transcriptional regulators, Pdr1 and Pdr3, respond to the activating compounds by inducing transcription of genes for ATP-binding cassette (ABC)-type efflux pumps, such as Pdr5, which has broad specificity. PDR can also be induced by mitochondrial dysfunction. The goal of the current study is to determine the PDR response to chloramphenicol, a drug that binds mitochondrial ribosomes, inhibiting translation in the organelle. *Saccharomyces cerevisiae* BY4741 was used as the model organism to begin these investigations. It was determined that Pdr1 and Pdr5 are key components of the PDR network involved in the high level of resistance that *S. cerevisiae* shows to chloramphenicol, and that transcription of some PDR genes is induced during exposure to the drug. Current studies are focused on the multidrug resistant state induced by chloramphenicol. *S. cerevisiae*[DC1] was primed with sub-inhibitory concentrations of chloramphenicol to activate the PDR pathway, followed by exposure to the clinically relevant drug fluconazole, which impairs ergosterol biosynthesis. Initial experiments suggest that activation by chloramphenicol does not provide resistance to fluconazole, even though both are substrates for the Pdr5 pump. Stress resulting from the combined actions of the two drugs may have led to this effect, and future experiments will address the impacts of mitochondrial function on induction of PDR.

Gen abstract type

Poster

Section Type

MGCM

136 - Spatial regulation and plasticity of bacterial cell elongation in the Caulobacteraceae

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Bacterial cell elongation requires precise spatial control of peptidoglycan (PG) insertion, yet the molecular mechanisms underlying this process, and how they diversify across species and conditions, remain poorly understood. Using the *Caulobacteraceae* family as a comparative framework, we identified striking diversity among closely related species: *Caulobacter crescentus* displays bidirectional midcell elongation, *Asticcacaulis excentricus* grows unidirectionally from midcell, and *Asticcacaulis biprosthecum* combines polar elongation with unidirectional midcell growth. These findings demonstrate that closely related bacteria can deploy distinct growth programs and that different modes of PG insertion can converge on similar overall cell morphologies. We further showed in *C. crescentus* that ecologically relevant temperature shifts reshape elongation patterns, suggesting that spatial modulation of PG synthesis may be a strategy for environmental adaptation.

Building on these discoveries, we are now investigating the molecular determinants underlying these distinct and environmentally responsive elongation programs. We are establishing CRISPRi platforms in *A. excentricus* and *A. biprosthecum*, enabling, for the first time, systematic genetic interrogation of cell elongation modes in these organisms. In parallel, we are using a high-throughput bacterial phenotyping platform together with transposon sequencing (Tn-seq) to screen mutant libraries across multiple conditions. Combined with quantitative fluorescence microscopy and fluorescent D-amino acid (FDAA) labeling, these approaches will allow us to identify factors controlling PG insertion and its plasticity under changing environmental conditions.

By defining conserved and species-specific determinants of cell elongation, this work will advance our mechanistic understanding of PG synthesis regulation and may uncover new vulnerabilities for antimicrobial development.

Gen abstract type

Poster

Section Type

MGCM

137 - Uncovering Atypical Evolutionary Patterns in Avian Pathogenic *Escherichia coli*

Megan Tomilin[1], Himen Salimizand[1], Chinenye Nnajide[1], Wolfgang Koester[1], Yejun Wang[2], Aaron White[1]

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Colibacillosis is a disease with great economic impact on poultry producers because it can affect all stages of poultry products. Colibacillosis is caused by a set of *Escherichia coli* strains referred to as avian pathogenic *E. coli* (APEC). Current methods of infection prevention have had limited success due to the extreme genetic diversity of APEC and antibiotic use is restricted due to antimicrobial resistance concerns. Currently, the distinction between pathogenic and non-pathogenic avian associated *E. coli* strains is also poorly characterized. A new method of targeting APEC strains is needed to mitigate future economic loss.

While many outer membrane proteins (OMPs) are conserved across Gram negative bacteria, mutations in the extracellular loops contribute to increased diversity. These mutated regions are referred to as hyper variable regions (HVRs) and typically face the external environment of the cell where they are accessible to phage and the host immune system. Phylogenetic studies of Gram negative bacteria have revealed HVRs as the result of mosaic evolution, wherein certain OMPs are evolving separately from the rest of the bacterial proteome. Furthermore, widespread recent bioinformatics analyses of diverse Gram negative species suggest there may be limitations to the variability of HVRs. We propose investigation of the HVRs in APEC strains may allow us to compensate for the variability in these conserved proteins providing a means of improving OMPs as targets for colibacillosis prevention and treatment.

We hypothesize that investigation of HVRs in avian associated *E. coli* strains can elucidate limitations of variability across pathogenic strains. We aim to: 1) Confirm patterns of mosaic evolution in the OMPs of avian associated *E. coli* strains. 2) Determine and compare the distribution of the HVRs in strains isolated from diseased and healthy birds. 3) Investigate the incidence of HVRs in pathogenic and non-pathogenic strains. We sequenced 245 avian associated *E. coli* strains from Saskatchewan broiler chicken farms to investigate mosaic evolution in real-world samples. Phylogenetic tree building was used to confirm incidences of mosaic evolution. Sequence alignment was used to identify previously established HVR sequences and establish the incidence of OMP variants. Another ongoing project used genomic analysis to predict likely pathogenic and non-pathogenic strains. Animal trials using broiler chicks were used to confirm virulence of APEC strains.

All our investigated strains demonstrated mosaic evolutionary patterns in the investigated OMPs. The atypical evolution of HVRs may contribute to *E. coli* evasion in the co-evolutionary arms race against host (avian) immune systems/phage/antibiotics. Only a couple variants of each OMP encompassed all the diversity within the entire avian associated *E. coli* data set. The restriction of the number of variants is likely due to the necessity of preserving function for these conserved proteins. Bias in HVR sequences between diseased and healthy birds and between pathogenic and non-pathogenic strains may suggest an approach to targeting diverse disease-causing strains with minimal impact to commensal bacteria. In the future, these analyses can also be applied to other *E. coli* pathotypes beyond APEC to elucidate possible correlations within other host organisms.

Gen abstract type

Poster

Section Type

MGCM

139 - RecA deletion prevents the evolution of effective nitrofurantoin resistance

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Antibiotic resistance poses a growing threat to public health, contributing to millions of deaths every year. Within this broader challenge, nitrofurantoin remains an important antibiotic in clinical use, making it especially relevant to understand how bacteria respond to its effects. One of the primary stress responses triggered by nitrofurantoin is the SOS pathway, which is activated by DNA damage. RecA plays a central role in this pathway by promoting DNA repair and mutagenesis. The importance of this response is underscored by the fact that RecA-deficient strains exhibit up to 64-fold greater sensitivity to nitrofurantoin, highlighting a strong link between the SOS response and nitrofurantoin susceptibility.

This study investigates RecA's role in the evolution of nitrofurantoin resistance and evaluates RecA inhibition as a therapeutic strategy. Using the Soft Agar Gradient Evolution (SAGE) system, we followed the evolution of resistance across 32 independent lineages of wild-type (WT) and Δ*recA E. coli*. We performed whole-genome sequencing to identify mutations associated with resistance, as well as lineages that failed to evolve higher levels of resistance. We found that Δ*recA* strains evolved significantly lower resistance levels than WT. We also found that canonical resistance pathways were not enough to achieve clinically relevant resistance levels in an SOS-deficient background. In addition, we identified alternative pathways that can contribute to nitrofurantoin resistance in the absence of a functional SOS response, along with the fitness cost associated with this evolutionary process. In parallel, we tested three reported RecA inhibitors for their ability to reduce nitrofurantoin resistance and found that chemical inhibition of RecA failed to reproduce the full hypersensitivity seen in genetic knockouts.

These findings reveal that targeting RecA represents a promising strategy to help preserve the clinical utility of nitrofurantoin and reduce the ability of bacteria to adapt during treatment. This work advances understanding of antibiotic resistance evolution and informs future approaches to constrain bacterial adaptation.

Gen abstract type

Poster

Section Type

MGCM

141 - Discovery of inhibitors of the *Pseudomonas aeruginosa* NADH:ubiquinone oxidoreductase (NQR) that hinder virulence factors

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Antimicrobial resistance has created an urgent need to identify novel therapeutic targets in bacteria. The NADH:ubiquinone oxidoreductase (NQR) is a potential target in a number of bacteria that transfers electrons from NADH to ubiquinone while pumping ions from the cytoplasm to the periplasm. In most species, this complex pumps sodium ions, whereas in *Pseudomonas aeruginosa* it pumps protons, thereby functioning as a member of the electron transport chain. Using a strain of PAO1 with the NQR knocked out, we demonstrate that the NQR complex plays a crucial role in the motility and biofilm development virulence factors in *P. aeruginosa*. We develop and execute a high-throughput inhibitor screen to identify and confirm compounds that inhibit NADH oxidation by this complex. Using single-particle cryogenic electron microscopy (cryoEM), we determine high-resolution structures of the NQR complex, both inhibitor-free and bound to one of the confirmed hits from our screen, demonstrating that it binds to the ubiquinone binding site. These structures provide insight into conformational dynamics controlled by binding at the ubiquinone site, with potential implications for the coupling between electron transfer and proton pumping in this complex. Biofilm development and motility assays with selected compounds from the screen show that they affect these virulence factors similarly to the NQR knockout.

Gen abstract type

Poster

Section Type

MGCM

144 - Adaptive laboratory evolution of *Yarrowia* isolates for improved utilization of polyethylene autoxidation products

Ameneh Norouzianghahfarokhi[1], George diCenzo[1]

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Polyethylene (PE) is the most widely produced plastic worldwide and a major contributor to persistent plastic waste because of its chemically inert carbon-carbon backbone and high resistance to biodegradation. One promising strategy to address this challenge is chemo-biological upcycling, in which PE is first chemically oxidized into smaller, more bioavailable compounds and then biologically converted into value-added products. Polyethylene oxidation generates a mixture dominated by dicarboxylic acids (DCAs), which may serve as bioavailable carbon sources for microbial growth. In this study, adaptive laboratory evolution (ALE) was used to improve the ability of two newly isolated *Yarrowia* strains, *Yarrowia* sp. Np-QU-20 and *Yarrowia* sp. No-QU-06, to utilize mixtures of PE autoxidation-derived DCAs. The two isolates were selected based on previous phenotypic screening showing strong growth on a wide range of DCAs. Each strain was repeatedly passaged through defined medium containing synthetic DCA mixtures designed to mimic PE autoxidation products. Parallel lineages were serially transferred over approximately 100 generations to select for improved fitness on these non-conventional carbon sources. Changes in growth performance were monitored throughout the evolution experiment by measuring optical density and comparing evolved populations to their ancestral strains. Phenotyping of the derived isolates indicated that ALE enhanced growth of both isolates on the DCA mixtures, with evolved lineages showing shorter lag phases and improved biomass accumulation relative to the ancestral strains. These findings suggest that *Yarrowia* populations can rapidly evolve to grow robustly on complex mixtures of plastic-derived substrates, and they support the feasibility of ALE as a strategy to optimize microbial hosts for plastic waste valorization. Ongoing work includes whole-genome sequencing to identify candidate adaptive mutations, and transcriptomic analysis to determine the metabolic basis of improved DCA utilization. Collectively, this work advances the development of *Yarrowia*-based platforms for sustainable chemo-biological upcycling of PE-derived waste streams.

Gen abstract type

Poster

Section Type

MGCM

149 - Systematic curation and enhancement of the MEGARes resistance gene database for AMR surveillance.

Uvindu Abeysekara[1, 2], Rodrigo Ortega Polo[2], Tim McAllister[2], Kim Stanford[1], Rahat Zaheer[2]

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With the rise of antimicrobial resistance (AMR), surveillance is extremely important. One method of surveillance is the use of genomic approaches using next-generation sequencing technologies. Databases of sequenced antimicrobial resistance genes (ARG) are integral to interpreting AMR at both the cellular (i.e., whole-genome sequencing) and environmental (i.e., metagenomic sequencing) levels. As ARG databases serve as references, it is extremely important that they are current and accurate. Errors in these databases can lead to false positives, such as identifying genes that do not confer resistance or misclassifying resistance mechanisms; or false negatives, where ARGs are absent or incorrectly annotated.

MEGARes v3.0 was generated by integrating 9 publicly available resistance gene databases, resulting in a comprehensive resource containing 8,733 ARG sequences. However, any errors inherent within the source database were incorporated into MEGARes. Curation errors were found to be of mainly two types: sequence-level and annotation-level. Sequence errors were generally minor and included, for example, reverse-complement representations of otherwise accurate coding sequences, incomplete gene coding sequences or excess flanking sequences. Annotation errors were more consequential, as they could lead to false-positive or false-negative calls. As an example, annotation-level errors included cases where sequences associated with resistance to one antimicrobial class (macrolides) were misclassified as conferring resistance to another (β-lactams), with direct implications for false resistance calls. Additionally, MEGARes 3.0 was last curated in 2022 and therefore needs updating.

Curation was accomplished by aligning MEGARes v3.0 against the latest individual databases to identify previously misannotated, missed, and new sequences. Additional curation involved removing excess flanking sequences and verifying that the annotation corresponded to a known ARG. Duplicate annotations were also removed. In total, 548 sequences with sequence errors and 328 sequences with potential annotation errors were identified, with their origin attributed to the source.

Once curation is completed, the original MEGARes v3.0 will be compared with the revised database using previously generated metagenomic sequence datasets derived from diverse sources, including cattle feces, agricultural and municipal wastewater, and soil, to evaluate the impact of curation on resistance detection outcomes. This study highlights the importance of rigorous database curation prior to sample analysis to minimize downstream analytical errors.

Gen abstract type

Poster

Section Type

MGCM

152 - Adapted Lab Evolution of the Legume Symbiont *Bradyrhizobium diazoefficiens* USDA110 for Low-Temperature Tolerance

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Nitrogen is crucial for plant health and agricultural productivity. Unfortunately, only 1-5% of the nitrogen in the soil is available to plants. Synthetic nitrogen fertilizers are often required to ensure sufficient crop yields; however, their application negatively impacts ecosystems. Using crops such as legumes, which can obtain nitrogen from other sources, is important for reducing our agricultural footprint. Most of the nitrogen needs of many legume crops in Canada are obtained through symbiotic nitrogen fixation by bacteria known as rhizobia. However, this process is hampered for soybean (*Glycine max*) crops in Canada due to low soil temperatures early in the growing season. Low soil temperatures limit the abundance and symbiotic associations of soybean rhizobia of the genus *Bradyrhizobium* due to their mesophilic nature. Low soil temperatures during the early part of the soybean season in Canada limit the soil proliferation of bradyrhizobia. In addition, low soil temperatures delay the formation of the symbiosis and onset of nitrogen fixation by weeks, reducing soybean yields. Here, *Bradyrhizobium diazoefficiens* USDA110 was grown in a common laboratory medium (yeast-mannitol broth) for approximately 200 generations under a low-temperature (15°C) condition using a serial passaging approach. After 200 generations, individual colonies were isolated to establish a collection of bradyrhizobia, each with random genetic variation. Growth curves indicate that most of the derived isolates exhibit an increase in fitness at both 15°C and 28°C relative to the ancestral *B. diazoefficiens* USDA110 strain. Whole-genome sequencing and variant calling of the derived isolates identified several cases of parallel evolution at both the gene and nucleotide levels. Mutations of interest will be further studied by introducing them into the ancestor. Transcriptomic sequencing will be conducted to elucidate any regulatory changes, and to further characterize mutations of interest. Lastly, the symbiotic efficiency of these derived isolates will be tested to establish if they confer an advantage to a soybean host under cold temperature. This work will advance our understanding of cold adaptation in bradyrhizobia and contribute to the development of inoculants adapted to Canadian soils. These inoculants could then be used by Canadian soybean growers to promote higher yields in an environmentally sustainable fashion.

Gen abstract type

Poster

Section Type

MGCM

156 - Antimicrobial resistance profiles of *Klebsiella pneumoniae* isolated from pediatric infections in British Columbia

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Background: *Klebsiella pneumonia* is a high-priority multi-drug resistant (MDR) pathogen causing community- and hospital-acquired infections, including pneumonia, urinary tract infections, bacteremia, liver abscesses, and meningitis. The highest rates of mortality due to infection have been reported in vulnerable populations such as neonates and young children aged under 5 years, older adults, and individuals with comorbidities, such as cancer or immunocompromise. The highest rates of infection are reported from low- and middle-income countries (LMICs). However, the prevalence of *K. pneumoniae* in Canada has been steadily rising and is a significant source of antimicrobial resistance (AMR) genes such as carbapenamases and extended-spectrum beta-lactamases (ESBLs).

Objective: To describe the prevalence of AMR genes and mutations in pediatric *K. pneumoniae* infections in Canada.

Methods: The RESTORE study (Surveillance of multi-drug RESisTant Gram-negative bacterial infectiOns in childREn) at BC Children's Hospital is an ongoing prospective study (September 1, 2019 to present), which includes participants aged ≤18 years with a Gram-negative bacteria isolated from blood, cerebrospinal fluid, urine, or other sterile site. Cases are excluded if polymicrobial growth is observed (including normal flora), or if samples isolated from urine didn't report associated symptoms of infection. If multiple isolates are collected during a single infection episode, one representative is included in downstream genomic analysis. Antimicrobial testing in the hospital clinical microbiology lab performed for *Klebsiella* species based on minimum inhibitory concentrations (MIC) against a standard panel of antimicrobials on the BD Phoenix™ M50 automated susceptibility platform (Becton Dickinson, MD, USA). Antimicrobial resistance profiles to designate MDR/non-MDR status were determined according to the clinical microbiology laboratory's susceptibility testing manual. For this study, isolates were further processed for whole genome sequencing using long-read sequencing on PromethION (Oxford Nanopore Technologies) to build high-quality genome assemblies, including reconstruction of plasmid assemblies not traditionally captured using short-read technology. On average, total genome length was 5.5Mb, assembled into 3 contigs with 90X coverage. Kleborate v3.2.4 was used to screen assemblies for determinants of AMR, including acquired genes and mutations conferring resistance. An overall resistance score was assigned based on the detection of ESBLs (=1), carbapenamases (=2), or carbapensamase and colistin resistance (=3).

Results: *K. pneumoniae* genomes (n=34) scored between 0-1 and no significant difference in scores was observed between urine (n=17) and blood (n=17) isolates. No carbapenemases were identified. The number of acquired AMR genes ranged from 0-13, conferring resistance against 8 classes of antibiotics. Commonly observed acquired AMR genes provided resistance against streptomycin (*strA, strB*, *aadA2*), beta-lactams ($bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1D}}$, $bla_{\text{OXA-1}}$), and sulfonamides (*sul1/2/3)*. In addition, AMR genes against fluroquinolones (*qnrA1/B1/S1*), trimethoprim (*dfrA12/A14/A27*), macrolides (*mphA, mrx*), and tetracyclines (*tetA/D*) were found at a lower frequency, followed by chloramphenicol (*floR, cml, catII*) and rifampin (*arr3*) with the lowest frequency. Chromosomal mutations conferring resistance against ampicillin were detected in 35% of isolates, and less frequently against fluroquinolones and colistin (12% and 6%).

Conclusion: Overall, acquired AMR genes and mutations were commonly observed in *K. pneumoniae* and further investigation of prevalence over time may reveal important patterns and guide treatment in children.

Gen abstract type Poster

Section Type MGCM

160 - Predicted 3D structures of *E coli* transcription factors and their DNA interactions

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The database of 3D protein structures predicted by Alphafold contains monomeric models of most of the proteins found in the UniProt database, including those in model organisms, such as *E coli* K12. Transcription factors (TFs), proteins that control gene expression, have a slight lower quality than other proteins. We therefore tested Alphafold 3, a version of the program that can predict multimeric proteins, as well as their interactions with DNA, to try and improve predicted TF structures, as well as test their predicted interactions with their DNA binding sites.

As testing subjects, we used a collection of TFs with several known target DNA binding sites, both with known, experimentally obtained, 3D structures, and without them.

Because most known TFs in *E coli* bind to DNA as homodimers, we produced TF models as monomers, for comparison against previously obtained predictions, since the database of freely available structures was built with Alphafold 2. We also produced homodimer models, as well as homodimers with up to ten target DNA sites, as well as ten non-specific sites.

Though we expected to see improvements going upwards from monomer to homodimer to homodimer+target DNA sites, predicted structures did not clearly show such a tendency, neither when compared with their experimental counterparts, when they were available, nor in terms of the confidence scores estimated by the program. It is possible that this is due to the monomeric models already being on high quality, even if lower than for most other proteins.

Predicted structures generally showed better interactions with their DNA target sites, compared to their interactions with non-specific DNA segments. Though sometimes the differences were not statistically significant, they still showed such differences. The models were produced with the target DNA site surrounded by extra nucleotides on both sides. Importantly, the program tended to put the proteins right on top of their target DNA sites. With non-specific DNA, the program put the protein anywhere along the DNA fragment. We therefore conclude that, as the software improves, these predictions will be useful for studying protein-DNA interactions specificity, when experimental structures are unavailable.

Gen abstract type

Poster

Section Type

MGCM

164 - Highly active in vitro reconstitution reveals insight into the regulation of the multidrug efflux pump MacAB-TolC

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Tripartite multidrug efflux pumps (TEPs) significantly contribute to the emerging threat of antimicrobial resistance in Gram-negative bacterial pathogens by exporting antibiotics from the cell. The study of these membrane protein complexes is particularly complicated as bacterial cells contain numerous TEPs with overlapping substrate specificity, making the study of their individual mechanisms a challenge *in cellulo*. To address this problem, we have developed a highly active purified *in vitro* membrane-reconstituted system to study individual ATP-driven TEPs. This *in vitro* reconstituted system enables us to monitor the substrate-stimulation of ATPase activity and transport of antibiotic substrates. Here we focus on one specific TEP, MacAB-TolC, that is implicated in tigecycline and macrolide resistance, along with biofilm formation, in the critical priority pathogen *Acinetobacter baumannii*. In addition, MacAB-TolC is involved in macrolide resistance and the transport of heat-stable enterotoxin II (STb), a virulence factor, from enterotoxigenic *Escherichia coli*. This TEP comprises three proteins: TolC, an outer membrane channel; MacA, a periplasmic adaptor protein; MacB, an inner membrane transporter that uses ATP-hydrolysis to fuel export. We therefore aim to investigate how functional partners, including lipids, substrates and adaptor proteins, influence this pump in a native-like *in vitro* reconstituted system to better elucidate the dynamics and mechanisms by which it operates. We demonstrate that both macrolides and tetracyclines, including tigecycline, stimulate the ATPase activity of *A. baumannii* MacAB. We also reveal, for the first time, that heat-stable enterotoxin II stimulates the ATPase activity of membrane-reconstituted *E.coli* MacAB indicating the versatile role of this TEP in both antimicrobial resistance and pathogenicity. Our study highlights the importance of allosteric regulation of this efflux pump by the periplasmic adaptor protein MacA, substrates and phospholipids, pointing at tight control of TEPs and mechanisms of resistance in bacteria. These exciting findings, along with our highly active *in vitro* reconstituted system, help to identify some key steps and conformations that are necessary for functional transport, bringing us a step closer to grasping how bacterial ATP-driven TEPs function as a whole. Ultimately, understanding the factors involved in the regulation of MacAB-TolC would allow us to discern how bacterial pathogens control and utilize their mechanisms of resistance, giving way to potential checkpoints that can be targeted therapeutically.

Gen abstract type

Poster

Section Type

MGCM

168 - Bacteriophages infecting *Acinetobacter pittii:* a genomic story

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Acinetobacter pittii (*Ap*) is a core member of the *Acinetobacter calcoaceticus-baumannii complex* alongside the virulent pathogens *Acinetobacter nosocomialis* and *Acinetobacter baumannii*. Whilst *Ap* is not considered a critical priority pathogen like *A. baumannii*, its prevalence in clinical infections is increasing, partly due to improvements in detection and identification methods. Isolation of *Ap* associated with hospital-acquired infections gradually increased in recent years. Isolates involved in hospital-acquired infections often carry genes conferring resistance to multiple antibiotics. Within the One Health framework, the convergence of an emerging nosocomial pathogen with significant environmental presence and expanding antimicrobial resistance, such as *Ap*, represents a critical threat to global health security. Whilst antibiotics are still generally effective against this pathogen, the global rise in antibiotic resistance, particularly carbapenem resistance, calls for the development of new therapies before current treatments become ineffective. The use of bacteriophages (phages) as a therapeutic tool represents a promising alternative to standard treatments. To develop such a therapy, there is a need to have a collection of isolated and fully characterized phages that infects a broad range of strains. To select optimal phage combinations for therapy, it is essential to characterize the genomics determinants of phage host range in both phage and bacterial genomes.

Our first objective was to isolate and characterize new phages with the goal of broadening the current global collection of phages infecting *Ap*. To do this, we used classic phage isolation methods using clinical isolates of *Ap*. We also characterized these phages by determining their host spectrum experimentally and by sequencing their genome. So far, we isolated 36 phages infecting clinical isolates of *Ap*. We determined the host spectrum of these phages using a collection of 22 clinical isolates of *Ap* and plaque assays. The hosts spectrum demonstrates that 10 of our phages have a broad host spectrum, infecting most of our strains. On the other hand, 26 phages have a more specific host range. Next, we sequenced bacterial and phage genomes through Oxford Nanopore, and the assembled bacterial genomes were analyzed using bio-informatics tools including FastANI, geNomad and PADLOC. Our results demonstrate that average nucleotide identity values for bacterial genomes ranged from 95% to 99% and that our strains contain on average 5 prophages and 22 phage defense systems per genome. This suggests there is substantial diversity within our *Ap* clinical isolates and that this diversity could correlate with phage susceptibility. Finally, the assembled phage genomes were aligned and clustered using bio-informatics tools including vConTACT2 and Clinker.

In perspective, we aim to identify the genomics determinants defining the phage host range in *Ap*. This would facilitate the rational selection of therapeutic phages based on specific infection profiles and genotypes. Ultimately, this work will enrich the collection of *Ap* phages and improve our fundamental knowledge on phage-host interactions in this bacterium. The long-term objective will be to develop an alternative therapy for this emerging multidrug-resistant pathogen.

Gen abstract type Poster

Section Type MGCM

170 - Phenotypic Screening and Characterization of Phosphatases in the Human Fungal Pathogen *Candida albicans*

Yunjin Lee[1], Linda Archambault[1], Vanessa Li[1], Sara Shariati[2], Faven Tesfamichael[3], Ci Fu[1], Nicole Robbins[1], Leah Cowen[1]

[1]University of Toronto, [2]University of Sydney, [3]University of British Columbia

Candida albicans is a common commensal fungus that can cause both superficial and deadly systemic infection, particularly in immunocompromised patients. Phosphatases are enzymes that catalyze the removal of phosphate groups and play key roles in cellular homeostasis and responses to environmental stress. While phosphatase function has been explored on a global scale in other fungal species, a systematic functional analysis of phosphatases in *C. albicans* has yet to be conducted. The Gene Replacement and Conditional Expression (GRACE) collection is a set of over 4,450 barcoded heterozygous deletion mutants in which the second allele has been placed under the control of a doxycycline (DOX)-repressible promoter. The recent addition of 1,240 GRACE strains expands the coverage of this mutant library to approximately 71% and includes all phosphatases and phosphatase regulators. Leveraging this library, systematic phenotypic screens were conducted to identify phosphatases important for fitness, susceptibility to azole and echinocandin antifungals, and morphogenesis, revealing several genes as important regulators of these diverse phenotypic traits. For example, Sds22, a putative regulatory subunit of the phosphatase Glc7, was identified as a novel regulator of caspofungin susceptibility in *C. albicans*. Follow-up experiments revealed that although both Sds22 and Glc7 positively regulate caspofungin susceptibility, Sds22 negatively regulates Glc7, as its overexpression blocked the Glc7-medited upregulation of the caspofungin-responsive gene, *PGA13.* This suggests a complex regulatory network by which Sds22 regulates the cell wall stress response, which is being further investigated. In addition, the RNA triphosphatase Cet1 was shown to be important for growth, antifungal susceptibility, and filamentation. While this large subunit of the Cet1-Cgt1-Abd1 mRNA capping complex played important roles in regulating these multiple phenotypes, genetic depletion of *ABD1* and *CGT1* had no impact on *C. albicans* filamentation, suggesting that Cet1 could possess previously undescribed biological functions that are important for governing the yeast-to-filament transition. Overall, this study implicates diverse *C. albicans* phosphatases as regulators of growth, antifungal susceptibility, and morphogenesis and highlights previously undescribed functions of these enzymes and their regulators that remain to be further explored.

Gen abstract type

Poster

Section Type

MGCM

173 - Elucidating the function and regulatory network of BBD18, an essential virulence-associated protein in *Borrelia burgdorferi*

Supriya Ramesh[1, 2], Satyender Hansra[1], Andrew Delainey[1, 2], Shu Hui Huang[2], Kerri Kobryn[2], Jenny Wachter[1, 2]

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Lyme disease, caused by the spirochetal bacterium *Borrelia burgdorferi*, is the most prevalent tick-borne illness in the Northern Hemisphere, with over 5000 cases in Canada in 2024, a ten-fold increase in incidence over the past decade. *B. burgdorferi* exists in an enzootic cycle transitioning between a hard Ixodes tick vector and a susceptible vertebrate host. Transmission between vertebrates and ticks requires precise control over gene expression and protein profiles to ensure survival. The changes from the incoming blood meal during tick feeding initiate a regulatory cascade in *B. burgdorferi* that results in the induction of the alternative sigma factor RpoS. RpoS controls the expression of numerous virulence-associated proteins essential for transmission, establishment and maintenance of vertebrate infection. Regulation of *rpoS*/RpoS occurs at both transcriptional and post-transcriptional levels, with BBD18 identified as a key repressor. Our previous work determined that BBD18 regulates not only *rpoS*/RpoS but also 14 additional genes and one small RNA. However, the mechanisms of BBD18 regulation remain unclear. To address this, we performed electrophoretic mobility shift assays (EMSAs) on DNA and RNA probes derived from the 5´ untranslated region (UTR) of *rpoS* and *bbk15* (one of 14 differentially regulated genes), along with protein/DNA/RNA pull-downs, and reporter assays. Our protein pulldowns and Western blots, along with previously published data, demonstrate that BBD18 is associated with the membrane. Our EMSA results indicate that BBD18 is unable to bind DNA and lacks nonspecific RNA binding activity but instead displays selective binding to the 5' UTRs of *rpoS* and *bbk15*. Reporter assays further demonstrate that the 5´ UTR of *rpoS* is sufficient for BBD18-mediated regulation of *mCherry* transcript levels, while BBD18 nucleic acid pulldown experiments show enrichment for RNA relative to DNA. Ongoing analysis of pulldowns will allow us to identify BBD18-associated transcripts and determine the sequence and/or structural determinants underlying target specificity. Together, these results demonstrate that BBD18 localizes to the membrane and regulates gene expression post-transcriptionally through RNA-binding, revealing a new layer of control in *Borrelia burgdorferi* adaptive gene regulation during transitions between vector and host.

Gen abstract type

Poster

Section Type

MGCM

176 - Characterizing phage-immunoglobulin interactions in healthy human guts

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Within the human gut microbiome, around 10^{10} viruses exist in equal abundance to bacteria. Among them, 97% are bacteriophages infecting bacteria rather than human cells. They lyse dominant bacterial strains, promote genetic diversity through horizontal gene transfer, thereby significantly shaping the microbial landscape along the GI tract. Enriched on the mucosa, these bacteriophages are constantly exposed to the immune-active environment, where immunogenic molecules such as immunoglobulins interact with multiple microbes to differentiate pathogenic from beneficial ones. However, whereas multiple studies have established the binding between immunoglobulins and commensal bacteria, little work has been done on these interactions with commensal bacteriophages. Here, we asked whether immunoglobulins bind commensal phages and whether we can develop a method to capture these interactions.

Prior work on immunoglobulin-bacteria interactions used either fluorescent-activated or magnetic-activated bacterial cell sorting, incorporated with 16S rRNA gene sequencing to identify IgA-coated bacterial taxa (IgA-Seq). This method, however, is unsuitable for viruses which lack a universal marker gene. A recent method called metagenomic immunoglobulin sequencing (MIg-Seq) was developed to bypass the need for marker gene enrichment. Building on this framework, I hypothesized that a similar strategy could be extended to viruses to study immunoglobulin-phage interactions. To address this, I developed IgVir, a method that adapts immunoglobulin-based magnetic separation from MIg-Seq but is specifically designed to enrich viral particles only. By integrating this enrichment with genome-resolved viromics, IgVir isolates and characterizes phages bound to IgA, IgM, and IgG into different fractions.

Using this approach, I processed fecal samples from 10 healthy volunteers. Preliminary results based on DNA quantification across IgA-, IgM-, and IgG-enriched viral fractions suggest successful enrichment of immunoglobulin-associated viral particles, with IgM+ viral fractions having the highest DNA concentration.

Although this work is ongoing and we are still awaiting metagenomic sequencing results, these findings indicate that commensal phages are bound by immunoglobulins and support IgVir as a viable framework to study these interactions.

Gen abstract type

Poster

Section Type

MGCM

177 - Sending mixed signals: exploring RstAB as a unique protein system in *Salmonella* Typhi

Josiah Lotecki[1], Casey Fowler[1]

[1]University of Alberta

Salmonella enterica subspecies *enterica* is an enteric bacterial pathogen that possesses a unique ability: it can persist within the cells of a human host, effectively hiding from the host's immune system and causing disease stealthily. My research focuses on the Typhi serovar: the causative agent of typhoid fever, which is responsible for 100,000 deaths annually. *Salmonella* Typhi produces sensor proteins to detect changes in its intracellular environment during infection, and cognate regulator proteins that control gene expression in response to the environment. Both proteins in these two-component systems increase the efficiency of *S.* Typhi's attack. The RstAB system in *S.* Typhi is of particular interest, where RstB is the sensor protein and RstA is the regulator protein. This protein system is essential for regulating the virulence of other enteric pathogens. However, *S.* Typhi demonstrates perplexing, uncommon interactions between its RstAB sensor and regulator proteins: while RstA/RstB predictably interact as partners in related intestinal pathogens like *Escherichia coli*, recent research has found that RstA does not require RstB to function in *S.* Typhi. This is a unique mutation that suggests novel, complex regulation in this pathogen. We suspect that *S.* Typhi's unique intracellular niche is a vital contributing factor to this difference, therefore, investigating this functional detachment between RstA and RstB will allow us to better understand the pathogenesis of *S.* Typhi. This research aims to utilize DNA mutations and sequencing, as well as protein analysis, to investigate the nature of the functionally detached relationship between RstA and RstB in *S.* Typhi. Potential other factors and sensor proteins that could be influencing these interactions will also be explored. The results of this research can play a vital role in understanding the nature of *S.* Typhi's intracellular pathogen-host interactions.

Gen abstract type

Poster

Section Type

MGCM

180 - Engineering and evaluation of polycationic nanopeptide-fused endolysins against *Mannheimia haemolytica*

Sidra Moqaddes[1], Hechao Du[2], Jieting Lin[1], Jae Eun Hyun[1], Mawra Gohar[1], Alexei Savchenko[1], Kim Stanford[3], Brenda Ralston[4], Tim A. McAllister[5], Dongyan Niu[1]

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Background Bovine respiratory disease (BRD), associated with multidrug-resistant (MDR) bacterial pathogens, remains one of the most important health challenges affecting the North American feedlot cattle industry. Among the pathogens involved, *Mannheimia haemolytica* is considered a major contributor. Current control strategies primarily rely on vaccination and antibiotics such as phenicols, tetracyclines, and macrolides. However, vaccine efficacy is often incomplete because of antigenic variation among strains and the complex, multifactorial nature of BRD. In addition, the continued use of antibiotics is increasingly limited by the rapid emergence of antimicrobial resistance, as well as growing regulatory and consumer pressure to reduce antibiotic use. These challenges emphasize the urgent need for alternative treatment strategies that can effectively target *M. haemolytica* while reducing the risk of further antimicrobial resistance development.

Endolysins are enzymes naturally produced by bacteriophages (phages) at the end of the lytic cycle to hydrolyze the peptidoglycan layer of the bacterial cell wall and release progeny phages from the host cell. Although endolysins have emerged as promising alternatives to antibiotics, their activity against Gram-negative bacteria is limited by the presence of the outer membrane (OM) barrier. However, the antibacterial activity of Gram-negative endolysins can be enhanced when they are engineered with OM-permeabilizing molecules such as polycationic nanopeptides (PCNPs). PCNP is short, highly positively charged peptide designed to interact with and disrupt the negatively charged cell envelope of Gram-negative bacteria, thereby facilitating the entry of therapeutic enzymes such as endolysins.

Objectives This study aimed to engineer prophage-derived endolysins and generate their PCNP-fused variants for evaluation as alternative antimicrobial agents against *M. haemolytica*.

Methodology In this study, five distinct prophage-derived endolysins from *M. haemolytica* were screened, and two of them (185 and 587AP2) were successfully expressed along with their engineered PCNP-fused variants (PCNP-185 and PCNP-587AP2). The endolysins were cloned into the pBAD plasmid and expressed in the *Escherichia coli* BL21(DE3) expression system. The recombinant proteins were then purified, quantified, and confirmed by SDS-PAGE prior to evaluation in muralytic (peptidoglycan degradation) and antibacterial activity assays.

Results All four recombinant proteins exhibited muralytic activity, with the original endolysins showing higher enzymatic efficiency. This finding suggests that PCNP primarily functions as an OM permeabilizer rather than as an enhancer of the intrinsic peptidoglycan-degrading activity of endolysins. In contrast, the PCNP-fused variants demonstrated greater antibacterial activity than their non-fused counterparts. Among them, PCNP-185 produced the most consistent multi-$\log_{10}$ reductions, achieving up to 4.5 decrease in bacterial counts across *M. haemolytica* serotypes, particularly when combined with EDTA, which further increased OM permeability through its chelating activity. In addition, the selection of an appropriate buffer was found to be critical for accurately evaluating antibacterial activity, as some buffers negatively affected bacterial growth and could lead to overestimation of endolysin-mediated antibacterial effects.

Conclusion and Importance These findings highlight the synergistic potential of PCNP-fused engineered endolysins and OM chelators for overcoming the Gram-negative OM barrier. To our knowledge, this is the first study to engineer and evaluate prophage-derived endolysins against *M. haemolytica*, underscoring their promise as innovative therapeutics for BRD-associated bacterial pathogens.

Gen abstract type

Poster

Section Type

MGCM

187 - Good luck or good listening? *Streptococcus thermophilus* senses and produces danger signals to limit phage infection

Christine Cerson[1], Rabia Fatima[2], Tejas Karrthik[1], Alexander Hynes[2]

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Bacteria can sense and communicate about cell density, nutrient availability, and antagonistic factors from other species, all of which are relevant to survival. Arguably the most important topic of bacterial discussion, though, is nearby cell lysis from viral predation by bacteriophages (phages). Excitingly, a growing number of phage-related danger signals are being discovered across species. In my work, I have encountered the ability of spent medium from *Streptococcus thermophilus* cultures to limit phage amplification. *S. thermophilus* is a commonly used bacterium for modeling phage infections. Strangely, it appears to survive phage challenge using a memory-based defense system, CRISPR (Clustered Regularly Interspaced Palindromic Repeats)-Cas, even in cases of no prior encounter with the phage to have formed such memory. There is almost certainly more to the story than luck - could these bacteria be surviving because of a danger signal that reaches them before the phage does?

To test this, I have grown *S. thermophilus* cells in a 1:1 mixture of fresh medium and cell-free spent medium from an *S. thermophilus* (self) overnight culture and subjected them to infection by either of two model phages, ϕ2972 and ϕ858. For both phages, titer was reduced (~10,000 fold and ~10 fold, respectively) in the presence of spent medium, compared to the untreated condition. Interestingly, exposure of *S. thermophilus* to spent medium from *Lactococcus cremoris,* a closely related bacterium, led to the same resistance outcomes as exposure to spent medium from self. I determined that phages are stable in these spent media, and that the protective agent(s) limiting phage infection is/are most strongly associated with spent media from stationary phase cultures.

With members of our lab, I expanded this work into *Pseudomonas aeruginosa* (gram-negative), *Brochothrix thermosphacta* (gram positive), and *Escherichia coli* (gram-negative) to explore whether *S. thermophilus* (gram-positive) also communicates danger to non-self species. We found no protection of *P. aeruginosa*, and only one phage-host pairing with a ~10 fold titer reduction in *B. thermosphacta,* suggesting that protection triggered by *S. thermophilus* spent medium is rare in other species. Surprisingly, in *Escherichia coli*, the majority of tested phages also amplified poorly (~10 to ~100 fold titer reductions) in *S. thermophilus* spent medium-exposed cells, while spent medium from *E. coli* had no protective effect on itself. Conversely, I found that *S. thermophilus* could be protected by exposure to spent medium from *E. coli* or *B. thermosphacta*, but always for only one of two model phages.

These findings highlight a danger signal in spent medium from stationary cultures of *S. thermophilus* that triggers phage resistance in self and non-self species. Thus far, *S. thermophilus* seems to be a stronger interpreter than communicator of danger, but an expanded range is needed to fully understand this network of protection. Presently, I am working to isolate the signals in spent media that alert *S. thermophilus* to potential danger, and to identify the form of resistance (such as CRISPR-Cas driven resistance) that ensues.

Gen abstract type Poster **Section Type** MGCM

188 - Biochemical characterisation of defense mechanisms of *Streptoccocus thermophilus*

Lia Normand[1], Zacharie Morneau[1], Audrey Leprince[1], Justine Lefrançois[1], Geneviève Rousseau[1]

[1]Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval

Streptococcus thermophilus is a lactic acid bacterium (LAB) widely used as a starter culture in the dairy industry for yogurt and cheese production. Bacteriophages (phages) are the most abundant biological entities on Earth and their presence in the environment is inherently linked to bacterial populations. In dairy facilities, lytic phages represent a major threat to milk fermentation processes. Infection of *S. thermophilus* by phages can induce cell lysis which may delay or even halt fermentation, leading to reduced product quality or complete product loss. Notably, the threat posed by phages in the dairy industry has been studied since the 1930s, highlighting the persistence and complexity of this issue. Given their ubiquity, the complete eradication of phages in dairy plants is likely impossible. Research efforts are therefore focused on controlling phage populations, particularly through the selection of bacteriophage-resistant LAB strains.

Our team has shown that *S. thermophilus* strains carry, on average, at least 7.5 defense mechanisms. Among these, the CRISPR-Cas systems are the most prevalent, being present in all strains. These systems are now widely exploited to develop phage-resistant industrial strains. However, the coevolution of phages and bacteria has not only expanded the diversity of bacterial defense systems, but has also driven the evolution of phage counter-defense mechanisms. *S. thermophilus* phages have evolved anti-CRISPR proteins (Acr) that enable them to evade CRISPR-Cas systems, as well as other proteins that facilitate escape from restriction-modification systems. Importantly, combining multiple defense mechanisms within a single strain has been shown to enhance phage resistance without compromising the bacterium's ability to acidify milk. These findings offer a promising approach to addressing the ongoing evolutionary arms race and support the exploration of additional defense systems.

Recently, our laboratory investigated various accessory defense systems, defined as those less prevalent in *S. thermophilus* strains. The genes encoding these systems were introduced into multiple *S. thermophilus* strains, and tested against sixteen phages belonging to the *Moineauvirus, Brussowvirus, Vansinderenvirus, Piorkowskivirus,* and *P738* genera. The objective of this study is to characterize the mechanisms of action of selected anti-phage systems.

For purification and *in vitro* analysis, these genes were cloned into the pET28a vector and transformed into different *Escherichia coli* expression strains. To date, 21 proteins have been explored. Eleven proteins have been successfully expressed, seven of which are found in the soluble fraction. Of these seven soluble proteins, five have been purified at high yields. These purified proteins have been the subject to a range of *in vitro* analyses, including stability assays, ligand binding studies, pull-down experiments, enzymatic assays and *in silico* structural analysis to better understand their roles in defense and to guide functional hypotheses. Crystallization trials for structural determination are also underway for some of these proteins of interest.

Characterizing these accessory defense mechanisms is essential to deepening our understanding of phage resistance and for strengthening industrial *S. thermophilus* strains through synergistic combinations of defense systems.

Gen abstract type Poster

Section Type MGCM

189 - Impact of chemotherapy and radiotherapy on gut microbiome in colorectal cancer patients: a scoping review

Annie Li[1], Alina Wang[1], Ahmer Karimuddin[1, 2]

[1]University of British Columbia, [2]St. Paul's Hospital

Background: As the third most common cancer in the world, colorectal cancer (CRC) has been extensively researched to develop improved treatments for patients. Chemotherapy and radiotherapy are among the most common CRC therapeutic treatment options, and have been previously shown to cause gut dysbiosis. However, current articles report mixed results on the effect of treatment on the gut microbiome in colorectal cancer patients, including variability in the microbial abundance, diversity and overall composition. Our goal is to assess the current literature and provide greater clarity on the effects of chemotherapy, radiotherapy and chemoradiotherapy treatment on the gut microbiome in CRC patients. Methods: We screened for all published literature from the MEDLINE database, excluding animal experiments and review articles. All forms of chemotherapy and radiotherapy were considered. The primary outcome is the abundance of different bacterial strains before and after chemotherapy, radiotherapy or chemoradiotherapy treatment for CRC patients. The secondary outcome is the quantitative and qualitative analysis indicating effects on patient quality of life. Findings: A total of 1097 records were screened for this scoping review. 15 studies were identified and extracted according to the inclusion and exclusion criteria. Of the included studies, 1 focused on neoadjuvant radiotherapy, 3 on neoadjuvant chemotherapy, 5 on neoadjuvant chemoradiotherapy, 4 on adjuvant chemotherapy, and 2 on co-symptoms developed as a result of treatment. Interpretation: Chemotherapy, radiotherapy and chemoradiotherapy before and after surgery were shown to generally decrease the abundance of the gut microbiome in CRC patients, while no statistical differences were observed for the diversity of bacterial composition. Consequently, the quality of life was significantly reduced as a result of treatment-induced gut dysbiosis, highlighting the need for further research on the effects of microbiome alterations on patient outcomes.

Gen abstract type

Poster

Section Type

MGCM

191 - Sensitive detection of *P. gingivalis* using multiplexed quantitative and digital PCR.

Benjamin Sinclair[1], Eric Bottos[1], Gwendolyn Freeze[1]

[1]Thompson Rivers University Department of Biological Sciences

The oral microbiome is a complex community of microorganisms that plays a critical role in maintaining oral health. *Porphyromonas gingivalis* is a bacteria often present innocuously in the oral microbiome but is also known to promote oral dysbiosis and is considered a 'keystone pathogen' of periodontal disease. In extreme cases, *P. gingivalis* can enter the bloodstream and increase susceptibility to cardiovascular disease. It can also pass the blood brain barrier, where it can promote formation of amyloid plaques associated with Alzheimer's disease. Its pathogenesis is driven by environmental factors, which lead to expression of a variety of virulence factors. Some major virulence factors are a class of cysteine proteases called gingipains, which are unique to *P. gingivalis* and are directly involved in tissue destruction and nutrient acquisition. Another species-specific virulence factor is HmuY, a hemophore-like heme binding protein, which is involved in iron/heme exploitation from the host. Methods for detection of *P. gingivalis* in oral samples are highly relevant given its clinical significance.

Previous research has used traditional and quantitative PCR methods to detect *P. gingivalis* but the targeting of virulence genes specifically, and the use of digital PCR (dPCR) for increased sensitivity, is limited. dPCR works by partitioning the sample into thousands of individual PCR reactions so that each partition contains 0-1 template molecules. Absolute quantification without a standard curve is attained by counting the number of partitions with successful amplification. This research developed a three-target multiplex assay to detect and characterize *P. gingivalis* on both qPCR and dPCR instrumentation. The assay uses primers and TaqMan probes targeting three species specific amplicons: the virulence genes *kgp* and *hmuY*, and the V2 region of the 16S rRNA gene. The gene *kgp* encodes a lysine specific gingipain, and a region non-homologous to other gingipains was targeted. The gene *hmuY* is established as a specific marker gene of *P. gingivalis*, along with its contribution to pathogenesis. The 16S rRNA gene was included as it is the most used species-specific marker for *P. gingivalis* and other species. All qPCR standard curves demonstrated strong linearity with $R^2 > 0.99$ for all targets in the multiplex. Minimal reduction in amplification efficiency from singleplex to multiplex was observed. Absolute quantification of *P. gingivalis* can be achieved from gDNA samples with high sensitivity using dPCR and increased robustness from the three targets. Insights into pathogenesis can be attained from cDNA samples, since *kgp* and *hmuY* both encode established virulence factors. The sensitivity of dPCR was significantly lower than qPCR, with detection down to 5 copies.

Gen abstract type

Poster

Section Type

MGCM

192 - A diversity-oriented small-molecule screen identifies novel azole potentiators with efficacy against drug-resistant fungal pathogens

Bradley Laflamme[1], Saif Hossain[1], Taylor Davie[1], Bonnie Yiu[1], Sara Fallah[1], Lauren Brown[2], Braden Martyn[1], John Porco[2], Nicole Robbins[1], Leah Cowen[1]

[1]University of Toronto, [2]Boston University

The rise of antifungal drug resistance in human-pathogenic fungi necessitates the development of novel treatment options. One promising approach to bolster the efficacy of established antifungals is through the use of combination drug therapies, where the impact of one antifungal is potentiated by the addition of a second molecule. Treatment with multiple compounds targeting distinct processes in fungi has the potential to effectively slow the evolution of antifungal resistance and restore drug sensitivity in drug-resistant strains. However, our understanding of the biological processes that can be exploited through chemical-chemical interactions in fungi - and how these interactions vary across distinct pathogenic fungi- remains quite narrow. Here, we present an antifungal development pipeline that combines small molecule screening, chemical-genetic profiling, and chemical-chemical interaction mining to identify compound combinations with efficacy against major fungal pathogens of humans. Using an expanded version of the diversity-oriented Boston University Center for Molecular Diversity (BU-CMD) library, we conducted a small molecule screen against a drug-sensitized strain of *Saccharomyces cerevisiae*, finding that 453 compounds in the library (11.1% hit rate) strongly abrogated growth. Most of these compounds had not been identified in previous BU-CMD screens performed against wild-type fungal pathogens, allowing us to identify a broad range of novel chemotypes with “cryptic” antifungal activity. We selected 39 priority compounds and performed both chemical-genetic profiling in *S. cerevisiae* and chemical-chemical interaction screening in various pathogenic fungi. These secondary screens enabled the identification of small molecules with predicted distinct mechanisms of action and favorable interactions with established antifungals. We highlight two compounds, CMLD012407 and CMLD012158, which we predict target actin organization and mitochondrial function, respectively, and which both potentiate the life-saving antifungal fluconazole against fluconazole-resistant *Candida spp,* including *Candida albicans* and *Candidozyma auris.* Intriguingly, both compounds antagonized fluconazole in fluconazole-susceptible isolates of *C. albicans*, suggesting that these compounds not only have distinct chemical-chemical interactions across different species but also across distinct isolates within the same species. Thus, our work uncovers molecules with novel antifungal activities, highlights the therapeutic potential of diverse compound combinations, and showcases the power of chemogenomic profiling to generate predictions on compound targets. Further dissecting how the evolution of drug resistance in pathogenic fungi can sensitize them to new drug combinations will ultimately unearth new vulnerabilities in pathogen populations for which our current treatment options fall short.

Gen abstract type Poster

Section Type MGCM

194 - Discovery of novel single channel chitoporins in *Vibrio* species

Oriana Robinson[1], Nikhil Thomas[1]

[1]Dalhousie University

Vibrio species are major contributors to global ocean carbon cycling by degrading plankton and copepod exoskeletons to support bacterial chitinolytic growth. Many studies have elucidated the protein transport and enzyme requirements for chitin oligosaccharide utilization. For example, a trimeric outer membrane porin, ChiP, is considered the primary and specific chitin oligosaccharide import mechanism for *Vibrio* species. Here, using an unbiased genetic fitness experimental approach (Tn-seq), we have discovered a novel porin (different from ChiP) that is required for efficient *Vibrio parahaemolyticus* chitinolytic growth. The porin is modeled to form a single channel beta-strand barrel structure and has features similar to the OprD family of bacterial outer membrane porins. *V. parahaemolyticus* null mutants deficient for the OprD-like porin exhibited a severe growth deficiency when colloidal chitin was the exclusive carbon source in the media. Genetic complementation restored chitinolytic growth to wild type levels thus revealing a vital and primary role for the single channel porin in chitin oligosaccharide import. Mass spectrometry analyses confirmed an outer membrane localization for the novel porin. Notably, this OprD-like porin is widespread, with conserved protein homologues in both environmental and pathogenic Vibrios, including *V. cholerae, V. vulnificus, V. parahaemolyticus, V. anguillarum*, and many others. These data suggest that numerous *Vibrio* species use an OprD-like porin (along with ChiP) to import oligosaccharides for chitinolytic growth. Importantly, the discovery of this single channel chitoporin reveals a new genetic fitness factor with relevance for environmental niche colonization in numerous *Vibrio* species.

Gen abstract type

Poster

Section Type

MGCM

195 - Cell wall hydrolysis drives a second wave of transpeptidation during cell separation in *Bacillus subtilis*

Vaidehi Patel[1], Yen-Pang Hsu[2], Maharshi Debnath[3], Michael VanNieuwenhz[4], Yves Brun[1]

[1]Université de Montréal, [2]Indiana University, [3]University of Massachusetts, [4]Baylor University

Bacterial cell division culminates in two sequential steps: septum formation and daughter cell separation. In Gram-positive bacteria such as *Bacillus subtilis*, septation produces a thick, multilayered peptidoglycan (PG) crosswall that must be cleaved to enable separation. While septum assembly is well understood, the mechanisms driving separation remain poorly defined.

We uncover a previously unrecognized second wave of transpeptidation that occurs after septum completion and coincides with septal PG hydrolysis. Unlike septal PG synthesis, this activity does not involve incorporation of new material, but instead remodels existing septal PG. We show that this process depends on PG hydrolysis by the autolysin LytF and requires the transpeptidase PBPH. Notably, PBPH localization to the septum is LytF-dependent, revealing tight coordination between hydrolytic and crosslinking activities.

Our findings define a dynamic remodeling phase that follows septation and is essential for efficient cell separation, uncovering a previously overlooked layer of bacterial cytokinesis.

Gen abstract type

Poster

Section Type

MGCM

197 - Phages and their ecological strategies to advance phage therapy

Theodore Josephs[1], Melissa Haines[1], Thomas Sicheritz-Pontén[2], Andrew Millard[1], Martha Clokie[1]

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The rise of antimicrobial resistance represents a major global threat, driving the need for alternative approaches such as bacteriophages. Bacteriophages are naturally occurring, highly diverse, and abundant viruses that target bacteria. Because they use unique strategies to take over their hosts and have narrow host ranges, multiple phages often need to be combined into cocktails for therapy.

Currently, therapeutic cocktail development is time-consuming and labour-intensive, focusing on host range testing and virulence. However, other beneficial traits important for successful host takeover are largely ignored and remain poorly understood.

This study aimed to address this knowledge gap by categorising phage traits into an ecological framework. Here, we repurposed Grime's CSR triangle, which classifies plants into competitors, stress tolerators, or ruderals (those with the ability to deal with continued disturbance), to classify phages based on their transcriptional phase, or whether they express the highest proportion of their genes in the early, middle, or late stages of infection. In the absence of robust gene function annotations for phages, this provides insight into which stage of the lytic cycle each phage dedicates significant resources.

We focused on phages within a urinary tract infection (UTI) cocktail targeting *Escherichia coli* and *Shigella sonnei*, both significant healthcare-associated pathogens. By integrating phenotypic, genomic, and transcriptomic data, this approach aimed to identify key functional traits associated with effective phage takeover.

Transcriptomic profiling of these UTI phages revealed distinct ecological strategies between two different genera (*Tequatrovirus* and *Phapecoctavirus*). *Tequatrovirus* phages exhibited a relatively balanced distribution of genes maximally expressed across the early, middle, and late phases. In contrast, *Phapecoctavirus* phages displayed highly skewed transcriptional regulation, with a predominant bias towards middle-phase gene expression. Further analysis of the core genes of three *Tequatrovirus* phages demonstrated strong temporal conservation, with 92% of those shared genes being maximally expressed in the same transcriptional phase. This work provides a foundation for the rational design of phage cocktails informed by ecological frameworks, which will help streamline novel cocktail development.

Gen abstract type

Poster

Section Type

MGCM

205 - Investigations on the Structure-Function Relationship of PatA from *Campylobacter jejuni*

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The bacterial cell wall is a highly organized structure, essential for maintaining cell shape, resisting osmotic stress, and preserving bacterial viability. Its main component is peptidoglycan (PG), a covalently linked polymer composed of alternating *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) residues joined by β-(1,4)-glycosidic bonds and reinforced by cross-linked peptides. PG has long been an effective antibiotic target because it is essential in bacteria and absent from eukaryotic cells. At the same time, it is vulnerable to host and bacterial lytic enzymes, and bacteria have evolved modifications to protect it from such attack. O-Acetylation of MurNAc residues is one mechanism by which bacteria protect PG from lysis, thereby promoting persistence and virulence. PG O-acetylation in Gram-negative pathogens like *Campylobacter jejuni* is mediated by PatA and PatB, but while PatB has been biochemically and mechanistically characterized, the structure-function relationship of PatA is largely unknown.

Previous studies have shown that wild-type *C. jejuni* exhibits a substantially higher level of PG O-acetylation compared to a *patA* deletion mutant strain (Δ*patA*), confirming that PatA contributes to efficient O-acetylation of the PG sacculus. However, functional characterization of PatA *in vitro* has been limited by the difficulty of purifying this integral membrane protein. To address this, we developed an *in situ* complementation-based assay that monitors the efficacy of engineered PatA mutants in protecting cells from EDTA-induced autolysis. By comparing turbidity loss over time of a Δ*patA* host complemented with wild-type *patA* to that of Δ*patA* complemented with site-directed mutant *patA* genes, we were able to discern the importance of targeted amino acids in the function of the enzyme.

We validated the assay by replacing the putative catalytic His315 and Tyr455 residues with Ala, which produced phenotypes closely resembling the autolysis-susceptible Δ*patA* strain, supporting their critical roles in catalysis. We then applied the assay to validate a structural model of PatA docked with the 3',5'-diphospho-adenosine component of its substrate acetyl-CoA. Trp274, Arg283, and Asn293 were each found to be required for PatA function, supporting the prediction that they bind the nucleotide at the entrance to a tunnel that extends through the enzyme from its cytoplasmic face to the periplasm. In contrast, Arg164 and Ser279, which are located near the tunnel but not predicted to bind substrate, were not found to be essential for activity. In addition to providing support for the structural model of PatA, these data indicate that the developed assay can distinguish the functional significance of specific amino acid residues. This provides an opportunity to further investigate acetyl transfer by PatA in Gram-negative bacteria and for exploring this pathway as an antivirulence target.

Gen abstract type

Poster

Section Type

MGCM

209 - Targeted modification of Staphylococcal cell wall glycopolymers by bacteriophage-derived enzymes

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Wall teichoic acids (WTAs) are a defining feature of the Gram-positive bacterial cell wall, known to play important roles in cellular processes including cell morphogenesis, cell wall synthesis, and antimicrobial resistance. As surface-exposed anionic glycopolymers, WTAs influence cell surface architecture and are involved in the expression of virulence properties by clinically significant pathogens such as *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA). Key to the pathology of *S. aureus* are its abilities to adhere to and colonize diverse biotic and abiotic surfaces, as well as its intrinsic resistance to host-deployed defences and its ability to grow in biofilms. These abilities are functionally linked to the presence of WTAs and are in part dependent on polymer modification, making WTAs attractive targets for direct-acting agents that can attenuate virulence properties.

WTAs are also critical receptors that mediate bacteriophage infection, and phages have been shown to harbour enzymatic machinery capable of degrading the significant amounts of WTA present in Gram-positive host cells to facilitate penetration across the cell envelope. Hence, we surveyed phage genomes for ORFs encoding putative proteins predicted to degrade poly(ribitol-phosphate) produced by most *S. aureus*, and aimed to investigate the effect of external depletion of WTA on bacterial physiology and virulence-associated properties. Using purified, recombinant proteins, we demonstrate that these enzymes - denoted T15 (from Rockefellervirus PH15) and T37 (from Staphylococcus phage 37) - catalyze the degradation of extracted WTA from *S. aureus* (ATCC 25293), as well as WTA in whole cells, representing the first description of enzymatic degradation of poly(ribitol-phosphate) WTA. Preliminary studies suggest that degradation efficiency is influenced by polymer modification (glycosylation, d-alanylation) and backbone composition ((poly) glycerol phosphate vs. (poly) ribitol phosphate), highlighting molecular determinants of substrate selectivity. Notably, such modifications are implicated in virulence, adaptive stress responses, and resistance to bacteriophage infection. We additionally examined the effect of external WTA removal on bacterial permeability to antimicrobial agents, and on biofilm formation. This work establishes WTA degradation as a novel approach to probe the bacterial cell surface without perturbing polymer biosynthesis, and provides a basis for further exploration of WTA-targeting strategies to suppress virulence.

Gen abstract type

Poster

Section Type

MGCM

211 - Evidence for a Distinct Intracellular Pathway for Wall Teichoic Acid Degradation in *Bacillus subtilis*

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Bacterial cells are encapsulated by a cell wall that prevents osmotic lysis, while maintaining cell shape and protecting against environmental insults. The main structural component of the cell wall is peptidoglycan (PG), but cell walls of Gram-positive organisms contain a second major component covalently attached to PG, wall teichoic acid (WTA). Typically composed of glycerol phosphate or ribitol phosphate repeat units, often modified with sugars or d-alanine, WTA mediates physicochemical properties and interactions of the cell envelope, and is critical for proper PG assembly and maintenance, as well as cell morphogenesis and cytokinesis. Although mechanisms underlying dynamic cell wall remodeling in the context of PG have been well-studied, analogous pathways for WTA degradation during vegetative growth are poorly understood. We surveyed the *Bacillus subtilis* genome for candidate genes encoding WTA degrading enzymes, and identified *yobO*, which encodes a protein sharing significant sequence similarity with the known WTA-degrading enzyme GP12 from bacteriophage φ.29. Additionally, the AlphaFold-predicted structure of the *yobO* gene product revealed a high degree of similarity to the GP12 crystal structure, including conservation of the active site architecture.

Here, using polyacrylamide gel electrophoresis-based assays and colorimetric assays for detection of inorganic phosphate, we demonstrate that recombinant, purified YobO protein catalyzes robust depolymerization of WTA isolated from *B. subtilis* 168. Substrate specificity studies, using WTA isolated from isogenic strains engineered to produce polymers lacking specific modifications, showed a departure from that of *B. subtilis* phosphate starvation-induced WTA degrading enzymes, GlpQ, and PhoD. In addition, high performance anion exchange chromatography was employed to analyze product distributions, enabling distinction between exo- or endo- modes of depolymerization and providing insight into processivity. To investigate cellular activity, we generated a strain expressing *yobO* from an ectopic locus (*amyE*) under the control of an inducible promoter (P_{xyl}) and assessed the effects of *yobO* upregulation on WTA polymer length distribution,

cell morphology and cell envelope-associate phenotypes. Notably, the YobO protein lacks an identifiable secretion signal peptide, further distinguishing it from GlpQ, and PhoD and suggesting intracellular action. Altogether, our findings implicate *yobO* as a previously uncharacterized regulator of WTA and offer new insight into the role of WTA remodelling and degradation in bacterial survival strategies.

Gen abstract type

Poster

Section Type

MGCM

214 - Uncovering MRSA susceptibility to antibiotics and the underlying molecular mechanisms in human plasma

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Staphylococcus aureus-associated bacteremia is a comprehensive public healthcare priority, accounting, at least, for 20% of bloodstream infection cases globally. Furthermore, limited therapeutic options worsen the clinical outcomes and highlight a crucial need for improved treatment approaches. One of the significant drivers of antimicrobial resistance of *S. aureus* is Methicillin-Resistant *Staphylococcus aureus* (MRSA) which is considered as a challenge in antimicrobial therapy. Microbiological diagnostics rely mainly on antimicrobial susceptibility testing (AST) using standard lab media, such as Mueller Hinton broth (MHB), which do not reflect the host-relevant conditions at the infection sites. To mitigate this constraint, we conducted ASTs using 50%(v/v) human plasma to simulate the infection-site conditions, comparing them with ASTs in MHB. We detected inconsistencies in ASTs with ≥ 2-fold shifts in seven of nine tested antibiotic classes in human plasma relative to MHB against MRSA USA300. For example, we detected an 8-fold increase in minimum inhibitory concentration (MIC) of daptomycin in human plasma relative to MHB against USA300. Among the most significant discordant results were an unexpected sensitization of the MRSA strain to β-lactam antibiotics, where we observed a 256-fold shift toward susceptibility to cefuroxime. High-throughput genomic screening and subsequent mechanistic characterizations were conducted to define the determinants of MRSA USA300 susceptibility to the β-lactam cefuroxime in human plasma, revealing cell envelope remodelling that include potential reduced penicillin-binding-protein-mediated peptidoglycan cross-linking and disrupted cell envelope stress response. Together, this work reveals mechanistic insights into bacterial antibiotic responses under host-relevant conditions. Further, the AST results described herein may guide optimized antibiotic therapy for MRSA bloodstream infections.

Gen abstract type

Poster

Section Type

MGCM

216 - Development of a High-throughput Genetic Screen to Identify Novel Adaptor Proteins

Casey Fowler[1], Isha Ghoorah[2]

[1]Principal Investigator, [2]Presenter

Two-component systems (TCSs) are essential regulatory mechanisms that enable bacteria to sense and respond to environmental changes by modulating gene expression. These systems are widely conserved among members of the Enterobacteriaceae, including *Salmonella enterica* and *Escherichia coli,* where they play critical roles in environmental adaptation, persistence, and host infection. Among these, the PhoPQ two-component system is a well-characterized system that regulates bacterial responses to stressors such as low magnesium, acidic pH, and antimicrobial peptides, thereby promoting intracellular survival and virulence. While canonical PhoPQ signaling involves a histidine kinase-response regulator pair, its activity is further fine-tuned by small adaptor proteins that can enhance or repress downstream gene expression.

Despite extensive study of PhoPQ, the full range of adaptor proteins that regulate this system remains incompletely understood. Many of these regulatory components are likely small, potentially lineage-specific proteins that have been overlooked due to their size and the limitations of traditional discovery approaches. Existing methods, such as transposon mutagenesis, have enabled the identification of some adaptor proteins but may be limited in efficiency and scalability. This highlights the need for alternative strategies to more effectively identify and characterize these regulatory elements.

To address this gap, this study aims to design and implement an overexpression-based screening method to facilitate the discovery of adaptor proteins that modulate PhoPQ activity. A genomic DNA library derived from *E. coli* is fragmented and cloned into inducible plasmids, which are then transformed into a reporter strain containing a PhoPQ-regulated *lacZ* construct. Upon induction, fragments encoding activating adaptor proteins are expected to enhance PhoPQ signaling, resulting in increased *lacZ* expression and darker blue colony phenotypes, whereas repressors will produce lighter colonies. This approach enables efficient identification and downstream sequencing of candidate regulatory elements.

By establishing a scalable and efficient screening strategy, this work provides a framework for uncovering previously uncharacterized adaptor proteins across bacterial species. Ultimately, this method has the potential to expand current understanding of TCS regulation and reveal the broader role of small regulatory proteins in bacterial signaling networks, with implications for targeting regulatory systems in pathogenic bacteria.

Gen abstract type

Poster

Section Type

MGCM

230 - Investigating the Role of Cell Surface Glycopolymers in Microbially Induced Calcite Precipitation by *Sporosarcina pasteurii*

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Sporosarcina pasteurii is a Gram-positive, spore-forming bacterium with high ureolytic activity that leads to the precipitation of calcium carbonate in its local environment, a process referred to as microbially induced calcite precipitation (MICP). Applications of MICP in construction and soil stabilization have long been recognized, particularly in the former given that the production and repair of conventional concrete, one of the most widely used materials worldwide, are costly and associated with a substantial carbon footprint. Thus, biocement production by *S. pasteurii* potentially offers a sustainable alternative. Although the genetics and enzymology of *S. pasteurii* ureolysis have been well characterized, fundamental gaps remain in understanding the role of the *S. pasteurii* cell wall in MICP, particularly the structures and surface architecture that facilitate calcium carbonate nucleation.

The cell walls of Gram-positive bacteria invariably comprise a thick peptidoglycan (PG) layer, with the covalently attached glycopolymer wall teichoic acid (WTA) as a second major component. Typically composed of glycerol phosphate or ribitol phosphate repeat units often modified with sugars or d-alanine, WTAs are critically involved in processes such as PG synthesis, cell morphogenesis, and cytokinesis. They additionally govern the physicochemical properties of the cell wall, as well as interactions at the cell surface including metal ion binding and homeostasis. However, the presence of WTA in *S. pasteurii* has not previously been described. Here, we analyzed the *S. pasteurii* (ATCC11859) genome and identified a complete set of putative WTA biosynthetic genes based on homology to known pathways in *Bacillus subtilis* and *Staphylococcus aureus*. After subjecting *S. pasteurii* cells to WTA isolation protocols, structural analysis by NMR revealed that *S. pasteurii* produces poly (ribitol phosphate) WTA with *N*-acetyl glucosamine modifications. Notably, d-alanine modifications were absent, consistent with the lack of identifiable homologs of genes encoding WTA d-alanylation machinery (dltABCD operon). We hypothesized that *S. pasteurii* WTA contributes to MICP by sequestering Ca^{2+} at the cell surface and tested this by employing chemical inhibitors of WTA synthesis and monitoring Ca^{2+} consumption in WTA-depleted *S. pasteurii* using an established colorimetric EDTA titration assay. The findings from this study provide new insight into the cell wall architecture of *S. pasteurii* and its role in MICP, offering a foundation for future synthetic biology approaches to optimize and control biocementation.

Gen abstract type

Poster

Section Type

MGCM

232 - Dissecting the Hmr Phosphorelay: Linking Environmental Sensing to Motility Control in *Caulobacter crescentus*

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The transition between motile and sessile states in *Caulobacter crescentus* is a tightly regulated process that enables adaptation to changing environmental conditions. This transition is primarily controlled by the second messenger cyclic di-GMP (c-di-GMP), where high levels promote adhesion and holdfast formation, while low levels favor motility and flagellum production. Previous work from the lab identified the HmrABCX pathway as a novel phosphorelay system that regulates this transition by controlling the proportion of cells that are motile versus those that commit to surface attachment. In addition, this pathway integrates environmental signals, including temperature and metal stress, into this developmental decision.

Although previous work establishes the Hmr pathway as an important regulator of motility and adhesion, key mechanistic questions remain unresolved. First, the mechanism by which environmental signals are sensed and transmitted remains unclear. In particular, how HmrC interacts with HmrA to initiate phosphotransfer and whether HmrC directly binds environmental ligands such as metal ions are unknown. Second, the downstream effectors that link the phosphorelay to changes in flagellum production and holdfast synthesis have not been identified. While the pathway is proposed to regulate c-di-GMP metabolism through enzymes such as DgcB and PdeA, this connection is inferred from phenotypes and has not been directly demonstrated.

This project aims to address these gaps by investigating both the input and output of the Hmr signaling pathway. To understand signal sensing, we will characterize the interaction between HmrC and HmrA using pulldown assays and mutational analysis of predicted sensory residues, including candidate metal-binding sites in HmrC and sensory domains in HmrA. Functional assays measuring motility, adhesion, biofilm formation, and flagellum production under different environmental conditions will be used to determine how these mutations affect signal transduction.

To identify downstream effectors, we propose that Cle proteins, a family of CheY-like response regulators known to bind c-di-GMP and influence flagellar behavior, act as outputs of the Hmr pathway. We have generated deletion mutants of cle genes individually and in combination with hmr pathway mutants, and will assess their effects on motility, holdfast formation, biofilm development, and the proportion of cells bearing a flagellum. Epistasis analysis will be used to determine whether Cle proteins function downstream of the Hmr pathway. Additionally, we will test the role of phosphorylation by introducing phosphoablative and phosphomimetic mutations in Cle proteins, particularly under stress conditions, to assess whether regulation occurs primarily through c-di-GMP or involves an additional phosphorelay-dependent layer.

Together, this work aims to provide a mechanistic framework linking environmental sensing to changes in cellular behavior in *Caulobacter*. By integrating phosphorelay signaling with c-di-GMP-mediated regulation, this study will advance our understanding of how bacteria fine-tune the balance between motility and adhesion in response to environmental changes.

Gen abstract type Poster

Section Type MGCM

234 - Development of genetic tools for the study of *Akkermansia* species.

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[1]University of Victoria

Akkermansia muciniphila is a mucin-degrading gut bacterium associated with metabolic health and healthy aging, making it an attractive candidate for therapeutic development. However, limited genetic tools have hindered our understanding of how *Akkermansia* species colonize the gut and degrade mucin. While we previously developed a genetic system for *A. muciniphila*, other *Akkermansia* species remain genetically intractable. Here we developed transposon mutant libraries in *A. massiliensis and A. muciniphila* subsp. *communis,* related species that are also associated with human health. Both libraries were created using a mariner transposon to generate random insertions in the genome. The *A. massiliensis*transposon mutant library has over 5,100 unique insertions covering 901 genes, as determined by transposon insertion sequencing. The mutants have also been arrayed for individual phenotypic analysis. Mutagenesis conditions for *A. muciniphila* subsp. *communis* have been established and library construction is underway. This study represents the first genetic manipulation of *A. massiliensis* and *A. muciniphila* subsp*. communis*, and will allow future research into their respective genomes, mechanisms of colonization and mucin utilization across *Akkermansia* species.

Gen abstract type

Poster

Section Type

MGCM

236 - Characterizing the impact of antibiotic sheltering on the growth of β-lactamase-producing bacteria in polymicrobial communities

Luca S. Petrella[1], Montserrat Mora-Ochomogo[1], Christopher T. Lohans[1]

[1]Queen's University

Due to the overuse of β-lactam antibiotics, resistance mechanisms such as β-lactamase enzymes have quickly arisen in bacteria to combat these antibiotics. These enzymes can hydrolyze and inactivate β-lactam antibiotics. In polymicrobial communities, β-lactamase-producing bacteria may inactivate β-lactam antibiotics before they affect susceptible bacteria, allowing these susceptible bacteria to survive. However, this antibiotic sheltering phenomenon could have a negative effect on the growth of resistant bacteria, as they are protecting their competition. We investigated how the replication of β-lactamase-producing bacteria is impacted by sheltering, exploring the effects of β-lactam antibiotic identity and concentration, as well as the identity of the β-lactamase that is produced.

Susceptible and resistant *Escherichia coli* BW25113 strains containing a pACYC184 plasmid were chosen as a model system. To differentiate between susceptible and resistant bacteria, we designed plasmids encoding fluorescent proteins to allow for easy differentiation between these populations during plate counting. The plasmid used for susceptible *E. coli* only contained the fuGFP gene, whereas resistant *E. coli* strains had genes encoding different β-lactamases (e.g., KPC-2, IMP-1) inserted into the plasmid along with different coloured fluorescent proteins (e.g. eforCP, mOrange). The susceptible and resistant strains were inoculated in growth media containing a β-lactam antibiotic and were cultured overnight. Afterwards the CFU/mL of both the resistant and susceptible bacteria were determined.

Our results reveal how antibiotic concentration influences the replication of β-lactamase-producing bacteria in polymicrobial environments. At lower antibiotic concentrations, colonies of β-lactamase producing bacteria were outnumbered by the colonies of susceptible *E. coli*. However, as the antibiotic concentration increased, the ratio of resistant colonies to susceptible colonies also increased until only resistant colonies appeared on the plates. This research into the factors that affect the growth of β-lactamase-producing bacteria could provide insight into the treatment of infections involving polymicrobial communities. Furthermore, this may inform on the evolution and dissemination of bacterial resistance mechanisms such as β-lactamases.

Gen abstract type

Poster

Section Type

MGCM

237 - Effects of Non-Canonical D-Amino Acids on BlaZ Activity and Antibiotic Susceptibility in *Staphylococcus aureus*

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[1]Queen's University

Antimicrobial resistance poses a major challenge for the treatment of *Staphylococcus aureus* infections. Susceptibility to β-lactam antibiotics is influenced by several overlapping resistance mechanisms, including altered penicillin-binding proteins, β-lactamase production, and changes in cell wall architecture. Many *S. aureus* strains produce the β-lactamase enzyme BlaZ, which inactivates β-lactams. Non-canonical D-amino acids are increasingly recognized as regulators of peptidoglycan remodelling in bacteria. Because peptidoglycan is a major component of the bacterial cell envelope, changes to cell wall structure caused by D-amino acids may alter β-lactam-associated phenotypes by affecting β-lactamase activity and antibiotic susceptibility. This study investigated whether exposure to selected D-amino acids alters BlaZ activity and antibiotic susceptibility of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA).

Nitrocefin hydrolysis assays were used to quantify BlaZ activity in MSSA and MRSA reference strains and clinical isolates following exposure to a panel of D-amino acids. Most BlaZ activity was associated with cellular fractions, with lower activity detected in cell-free culture supernatants. Among the D-amino acids tested, D-Trp produced the greatest increase in BlaZ activity in both MSSA and MRSA reference strains, whereas D-Met produced a smaller, but detectable effect. Fractionation experiments further showed that the D-Trp-associated increase was greatest in the cell-containing samples, while cell-free culture supernatants showed a similar magnitude of increase. Analysis of MSSA and MRSA clinical isolates showed substantial heterogeneity in both baseline BlaZ activity and D-Trp responsiveness.

To determine whether these phenotypic changes were accompanied by altered antibiotic susceptibility, broth microdilution and checkerboard assays were performed. As expected, MSSA and MRSA reference strains displayed distinct baseline susceptibility profiles, particularly for β-lactams. In checkerboard assays, D-Trp most often produced additive rather than strongly synergistic interactions, whereas D-Met produced more synergistic effects in selected combinations, especially when paired with meropenem.

Together, these findings show that D-amino acid exposure increases BlaZ activity in both MSSA and MRSA. When combined with antibiotics, D-amino acids also produced overall additive or synergistic effects on bacterial responses. The data support a potential model in which D-amino acids perturb the staphylococcal cell envelope in ways that may alter BlaZ activity or regulation and, in some contexts, the susceptibility of *S. aureus* to antibiotics. Although the precise mechanism remains unresolved, this study provides a foundation for further investigation into how D-amino-acid induced peptidoglycan remodelling may influence β-lactam efficacy in *S. aureus*.

Gen abstract type Poster

Section Type MGCM

239 - Species-specific Structural Diversity of Lipid A in *Akkermansia*

Arianna Lagoudakis[1], Silas Porter Crenna[2], Angela M. Jackson[2], Kenneth Weke[2], Georgia Tiegs[1], David Goodlett[1, 2], Helena Petrosova[2], Lauren Davey[1]

[1]Department of Biochemistry and Microbiology, University of Victoria, [2]University of Victoria Genome BC Proteomics Centre

Akkermansia species are important members of the intestinal microbiota and key modulators of immunological and metabolic health. These Gram-negative commensals successfully colonize the gut without inducing inflammation, a process that remains poorly understood given the pro-inflammatory potential of surface lipopolysaccharide (LPS). In contrast to Gram-negative pathogens, whose LPS robustly activate Toll-like receptor 4, commensal bacteria often exhibit LPS variation that dampens immune activation, including loss of the O-antigen and production of lipooligosaccharide (LOS). *Akkermansia* species vary in their ability to activate Toll-like receptors, but the structural basis for this remains unclear. To investigate LOS diversity, we used Fast Lipid Analysis Technique to analyze the lipid A in a collection of *Akkermansia* strains and species. All *Akkermansia* strains examined produced lipid A containing phosphoethanolamine (pEtN) modifications. The abundance of pEtN-modified lipid A varied by species and across growth phases, which correlated with survival to colistin exposure. The presence of pEtN-modified lipid A, which has been linked to antimicrobial resistance, suggests a mechanism for bacterial survival and persistence in the gut. These results highlight species- and growth phase-specific differences in lipid A structure and identify the pEtN modification as a variable feature that may contribute to immune modulation and successful gut colonization.

Gen abstract type

Poster

Section Type

MGCM

247 - In search of population-wide responses to phage infections in *Streptococcus thermophilus*

Niousha Fazeli[1], Alexander Hynes[1]

[1]McMaster University

Bacteriophages, viruses that infect bacteria, exert strong selective pressure on bacterial survival. In response, bacteria have developed diverse antiviral defense systems, often involving transcriptional reprogramming that can extend beyond the infected cells and drive population-level responses. Previous transcriptomic study of *Streptococcus thermophilus*, a key species in yogurt fermentation and the dairy industry, showed that within only 20 minutes of infection, the RNR operon exhibits the strongest transcriptional response. This change is detectable even when only approximately 30% of cells are infected, with population-level transcript levels reaching 8-fold higher than in uninfected cells. Ribonucleotide reductases (RNRs) are the only class of enzymes that produce DNA building blocks (dNTPs) *de novo*. Therefore, they play a central role in nucleotide metabolism, DNA replication, and repair. I hypothesize that this upregulation occurs in the uninfected cells and is triggered by signals released from neighboring phage-exposed cells. Bacteria can communicate and coordinate population-level responses to environmental changes. One such mechanism is quorum sensing (QS) which is mediated by small molecules or peptides. In this ongoing work, I aim to isolate this potential signal by filtering spent media from phage-bacterial interactions and assessing its effect on RNR regulation in uninfected cells. Preliminary results show no significant upregulation in cells treated with filtrates from infected cultures, potentially due to signal dilution during processing. Current efforts include the use of a lux reporter system, microscopy, and testing a range of MOIs (multiplicity of infection) to determine whether cells respond to phage-infected neighbors. This response may have important consequences for both bacterial populations and phage infection. Given their role, RNR upregulation may represent a stress response that contributes to phage defense or conversely, a communication-mediated mechanism that prepares neighboring cells for infection, enhancing phage replication. These findings may reveal a previously uncharacterized communication-mediated mechanism in *S. thermophilus* in response to phage infection, highlighting microbial population dynamics and phage-host interactions.

Gen abstract type

Poster

Section Type

MGCM

248 - Experimental Evolution of the Lysis-Lysogeny Switch in Bacteriophage HK97

Eve Adams[1], Alexander Hynes[1]

[1]McMaster University

Bacteriophages (phages) are bacteria-specific viruses that can undergo either lysogeny, a stable association with their host, or lysis, which kills the host to release new progeny. The decision is controlled by a genetic switch that responds to environmental cues such as DNA damage in the host, triggering induction from lysogeny to lysis. Although natural phage populations show variation in their sensitivity to induction signals, it remains unclear how readily this genetic switch can evolve and what genetic changes underlie shifts in induction sensitivity. In this study, I experimentally evolved the model *Escherichia coli* phage HK97 to modify its responsiveness to ciprofloxacin-induced DNA damage. I exposed lysogens to ciprofloxacin and used phages released by induction to form the next generation of lysogens with the goal of enriching for hypersensitive variants over time. In a parallel selection approach, I retained only cells that did not induce during ciprofloxacin exposure and isolated phages released later through spontaneous induction, aiming to enrich for variants hyposensitive to DNA damage-triggered induction. Across both selection regimes, phage populations evolved increased induction sensitivity, with evolved lineages exhibiting a 1.5-2 log increase in titer following ciprofloxacin treatment relative to the ancestral phage. In the hypersensitivity lineages, this shift occurred rapidly within three passages and plateaued thereafter. Unexpectedly, similar increases were observed in lineages evolved under the hyposensitivity protocol, indicating that selection outcomes were not strongly differentiated between regimes. Additionally, spontaneous prophage induction remained largely stable across passages, although some lineages showed elevated baseline induction, particularly those passaged without antibiotic exposure. Together, these results demonstrate that induction sensitivity in HK97 can increase rapidly under laboratory passaging, while also revealing constraints on the ability to direct this trait in opposing directions. Ongoing whole-genome sequencing of ancestral and evolved populations will identify mutations associated with these phenotypic changes and reveal how HK97's regulatory circuit is modified during evolution. This work provides an experimental framework for studying how viral genetic switches balance stability with responsiveness.

Gen abstract type

Poster

Section Type

MGCM

250 - A Single Hcp Scaffold Recruits Diverse Effectors to the H3-Type VI Secretion System

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The Type VI secretion system (T6SS) is a specialized protein apparatus used by many Gram-negative bacteria to deliver toxic effector proteins directly into neighbouring cells. By targeting competing bacteria and host cells, T6SSs facilitate interbacterial competition and host virulence. Delivery of effector proteins to target cells can be accomplished by covalent fusion to, or non-covalent interaction with, major structural components of the apparatus. *Pseudomonas aeruginosa*, a clinically significant opportunistic pathogen, encodes three distinct T6SSs. Of these, the H3-T6SS is the least characterized but is implicated in host-specific virulence, a function rarely demonstrated for T6SSs. Recently, we identified a collection of structurally and functionally diverse host-targeting effectors from the H3-T6SS of *P. aeruginosa* that are encoded within a genomic recombination hotspot. These effectors are not fused to structural components of the T6SS, suggesting they rely on non-covalent interactions with the highly conserved secretion apparatus for delivery. Consistent with this, a recent structural study identified interactions between two H3-T6SS effectors and Hcp3, the tube component of the H3-T6SS. Building on this approach, we co-expressed tagged Hcp3 with each of the identified H3-T6SS effectors and performed co-immunoprecipitation and size-exclusion chromatography to assess complex formation. All of the identified effectors (10 total) demonstrated varying degrees of interaction with Hcp3, confirming their association with the inner tube protein and supporting an Hcp3-dependent mechanism of secretion. Bioinformatic investigation using Hcp3 as a probe identified homologs across phylogenetically diverse Gram-negative bacteria. Interestingly, amino acid sequence conservation across Hcp3 homologs is concentrated at the inner tube lumen, raising the question of how a single, highly conserved scaffold accommodates structurally diverse effectors. Furthermore, effectors from these diverse systems exhibited toxicity in a yeast model system, and structural models showed similarity to characterized host-targeting virulence factors, providing functional insight into H3-T6SS-associated effectors beyond *P. aeruginosa*. The consistent host-targeting activity observed across phylogenetically distant H3-T6SS systems suggests that eukaryote-specific effector delivery may be a defining and evolutionarily conserved feature of the H3-T6SS. Together, our findings identify Hcp3 as a versatile recruitment platform for structurally diverse H3-T6SS effectors and suggest that Hcp3-mediated loading represents a broadly conserved strategy for H3-T6SS effector delivery.

Gen abstract type

Poster

Section Type

MGCM

255 - Approaches to isolating Neisseria gonorrhoeae phages for fighting multidrug resistant (MDR) gonorrhea infections

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Antimicrobial resistance (AMR) is rising at an unprecedented rate and continues to threaten public health globally. In 2019, AMR contributed to approximately 5 million deaths, and this number is estimated to rise if AMR is not halted in bacterial pathogens. *Neisseria gonorrhoeae*, the human pathogen that causes gonorrhea, has developed resistance to almost all antibiotics that were once used to treat it, including the currently recommended treatments ceftriaxone and azithromycin. *N. gonorrhoeae* strains resistant to multiple classes of antibiotics are referred to as “superbugs,” causing infections that are potentially untreatable. Gonorrhea is the second most common sexually transmitted infection (STI), with 82.4 million cases reported in 2020. However, with 40% of infections being asymptomatic in men and 50-80% of infections being asymptomatic in women, this number is estimated to be much higher. When left untreated, gonorrhea can lead to infertility, pelvic inflammatory disease, ectopic pregnancy, and disseminated gonococcal infection. Thus, it is crucial that new antimicrobial therapies are identified for treating gonorrhea. A promising alternative treatment to antibiotics is phage therapy, which utilizes bacterial viruses (phages) to target and kill susceptible cells. Phages are classified as either lytic or lysogenic based on their lifecycle. Lytic phages infect their host to generate new phage particles that rupture the cell and are released into the environment. Alternatively, lysogenic phages can integrate their genome into host bacterial genomes as prophages and persist there until an environmental stressor causes them to enter back into the lytic cycle in a process known as induction. The increased availability of bacterial genome sequences in databases has made it possible to search for lysogenic phages, which is advantageous for organisms like *N. gonorrhoeae* that are difficult to grow in the laboratory. To date, complete prophage genome sequences have been identified in several *N. gonorrhoeae* strains. In one approach to induce these prophages back into the lytic cycle, inducing agents such as mitomycin C or hydrogen peroxide were used in an attempt to produce new phage particles that can be isolated. In a second approach, since *N. gonorrhoeae* colonizes the genitourinary system and phages are likely to be found wherever their host is located, it was anticipated that *N. gonorrhoeae* phages shed via bodily secretions could be isolated from wastewater samples. Though few positive phage isolations have been achieved, our results from these experimental approaches are presented and discussed.

Gen abstract type

Poster

Section Type

MGCM

256 - Genomic Insights into *Akkermansia muciniphila*'s Response to Bile Acids

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Akkermansia is a common gut microbe unique in its ability to utilize mucin as its sole carbon and nitrogen source. Bile acids, particularly ursodeoxycholic acid (UDCA), increase *Akkermansia*'s abundance despite their detergent properties. The genetic mechanism underlying this effect remains unknown due to the genetic challenges the bacterium presents. Here, we screened a transposon mutant library to determine the mechanistic basis of *Akkermansia*'s growth in the presence of UDCA. From this initial screen, three groups of mutants were identified with distinct responses to UDCA compared to wild type: (i) mutants whose growth is rescued by UDCA, (ii) mutants that develop a growth defect, and (iii) mutants whose growth remains unchanged. Analysis of these mutants provides insight into how UDCA modulates *Akkermansia*. One example is *amuc*_0395::Tn, which has a growth defect in mucin that is rescued by UDCA supplementation, indicating altered mucin utilization. Further growth assays revealed that UDCA supplementation rescues growth in the absence of N-acetylglucosamine (GlcNAc), a key component of mucin, suggesting that UDCA can bypass the requirement for this cell wall precursor. Understanding the mechanism behind *A. muciniphil*a's response to UDCA will provide insights into how gut metabolites modulate its colonization and the broader ecosystem.

Gen abstract type

Poster

Section Type

MGCM

258 - Tracking cellular responses and growth inhibition of *Salmonella enterica* and *Enterococcus faecalis* exposed to common SSRIs under anaerobic conditions

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Selective Serotonin Reuptake Inhibitors (SSRIs) are the most commonly prescribed class of antidepressants, but their impacts on native gut bacteria are not well understood. SSRIs inhibit the bacterial growth in lab culture. Most studies test bacterial responses to SSRIs in high oxygen (atmospheric) conditions, but the human intestine is a low-oxygen environment, ranging from 0-3 % O_2. We measured growth inhibition of facultative anaerobes *Salmonella*, a true pathogen, and *Enterococcus,* a common resident of the human large intestine, by the two most frequently prescribed SSRIs, sertraline and fluoxetine, in micro-aerobic conditions. *E. faecalis* was more susceptible to both SSRIs than *S. enterica*. Minimum inhibitory concentrations (MICs) for sertraline and fluoxetine were determined to be 250 mg/L and 200 mg/L, respectively, for *S. enterica*, and 150 mg/L of either SSRI for *E. faecalis*. The transcriptional response of *S. enterica* serovar Typhimurium SL1344 after 60 minutes of exposure to subinhibitory concentrations of SSRI exposure was examined using RNA-seq. The transcriptional responses were surprisingly minimal. Both SSRI treatments stimulated expression of the *torACD* operon, perhaps due to the presence of DMSO as an SSRI solvent. Only fluoxetine induced expression of the genes *asr* and *ydgU*, both coding for hypothetical stress response proteins. The reactive oxygen species (ROS) responsive genes *katG* and *soxS* were upregulated along with efflux pump regulators. Conversely, SSRIs consistently repressed the stochastic activation of Salmonella Pathogenicity Island 1 genes in exponential growth. These RNA-seq findings are the first to interrogate the rapid adaptive response of a bacterial species to SSRIs and reveal discrete responses to pharmaceutically relevant doses of two common SSRIs.

Gen abstract type

Poster

Section Type

MGCM

262 - Investigating the potential anti-fungal properties of the bacterial genus *Pantoea*

Adria Bateman[1], John Stavrinides[1]

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Candida albicans is the most prevalent fungal species that colonizes the human microbiota. Typically commensal, polymorphic *C. albicans* is maintained in its yeast form in the oral cavity, gastrointestinal and vaginal tracts of the normal flora by microbial competition, host immunity, and environmental factors. However, *C. albicans* can act as an opportunistic pathogen under conditions of microbiome dysbiosis, which can facilitate a morphological transition to its multicellular hyphal form resulting in invasive candidiasis.

Treatment of hyphal *C. albicans* is challenging due to the limited number of unique fungal targets, narrow range of available therapeutics, and its ability to invade the host immune system. Additionally, the rise of antimicrobial resistance (AMR) from antimicrobial overuse poses a serious global threat to public health and agricultural industries. As the evolution of multi-drug resistant (MDR) pathogens like *C. albicans* surpasses rates of novel antimicrobial discovery, resistant infections are becoming increasingly difficult to treat, making the discovery of novel antimicrobials critical.

The bacterial genus *Pantoea* produces a variety of antimicrobials that are effective against MDR pathogens and a promising source of clinical and agricultural therapeutics. In total, 22 antimicrobial natural products encoded by 12 biosynthetic gene clusters (BGCs) have been described in *Pantoea*. Most *Pantoea* natural products are antibacterial, with some strains exhibiting antifungal properties.

Pantoea agglomerans E.2012-30 is a strain isolated foxtail barley that prevents hyphal development in *Candida albicans* ATCC 10231. Transposon mutagenesis was used to create a mutant knockout library, which was screened for mutants that no longer inhibited hyphal development. A putative twelve-gene biosynthetic gene cluster, PNP-6, was identified, which included a FdhF/YdeP family oxidoreductase, metallophosphoesterase, MBL fold metallo-hydrolase, LysR family transcriptional regulator, MFS transporter, α/ β hydrolase, efflux RND transporter permease subunit, and a methyl-accepting chemotaxis protein among several hypothetical proteins.

The cluster is localized on plasmids in approximately 50 strains of *Pantoea* and is overrepresented in *Pantoea agglomerans* in comparison to other *Pantoea* species. Knockout mutants screened against a panel of Gram-negative human pathogens revealed that the cluster does not exhibit antibacterial activity. However, the E.2012-30 strain produces another antimicrobial that is synthesized by a separate BGC. This study provides insights into the understudied interactions between *Pantoea* and fungal pathogens, and the identification of potential antifungal natural products with therapeutic potential.

Gen abstract type Poster

Section Type MGCM

263 - Pathogenesis and antimicrobial susceptibility of *Burkholderia cenocepacia* in a macrophage model.

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Burkholderia cenocepacia (Bce) is an opportunistic respiratory pathogen that causes devastating and often untreatable infections in people with cystic fibrosis (CF). Chronic infections from pathogens such as Bce are the leading cause of death in CF patients, often leading to lung failure and the fatal “cepacia syndrome”. This clinical challenge is caused by high levels of intrinsic antimicrobial resistance and is compounded by the CF lung environment. The hallmark accumulation of stagnant, thick mucus in conjunction with distinct nutritional and environmental characteristics favours the development of chronic Bce infections. Further complicating treatment, clinical evidence from CF lungs postmortem suggests that Bce does not form a biofilm in the CF lung, as was previously assumed, but rather primarily resides within macrophages. We aim to understanding how this intracellular lifestyle contributes to pathogenesis and antimicrobial susceptibility, and to identify antimicrobials that better target bacteria in this context. We have developed a RAW264.7 macrophage infection model compatible with live-image high-content screening to monitor infection efficiency and intracellular bacterial proliferation over time. This approach has allowed for the first studies on the efficacy of antimicrobials and bioactive compounds against intracellular Bce. The advantages of this approach are that it uses wild type bacteria, does not require antibiotics to control extracellular growth, and can be used to monitor an infection’s progression over time. Using this methodology, we demonstrated that intracellular Bce are significantly protected from antibiotic-mediated killing compared to their extracellular counterparts. Intracellular Bce survive and replicate despite concentrations of antibiotics that are lethal *in vitro*. Furthermore, we have screened a library of ~700 compounds and have identified two classes of antibiotics with greater efficacy than the clinical standard at clearing intracellular bacteria. Considering that results from traditional drug screening performed in liquid culture vary in their ability to translate to clinical efficacy, our results indicate that an intracellular lifestyle may contribute to this discrepancy.

Gen abstract type

Poster

Section Type

MGCM

271 - Dissection of the *Klebsiella pneumoniae* type two secretion system through gene deletion and functional characterization

Eric Toombs[1], Jenny-Lee Thomassin[1]

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Klebsiella pneumoniae are bacteria found ubiquitously in nature and can cause infections in humans, animals, and some plants. These bacteria are increasingly becoming antibiotic resistant and in 2024, the World Health Organization (WHO) listed carbapenem-resistant and third-generation cephalosporin resistant *K. pneumoniae* as priority number 1 and number 6 for research into therapies and vaccine development. Type two secretion systems (T2SS) are protein nanomachines used by various species of bacteria, including *K. pnuemoniae*, to secrete proteins that play key roles in infection and environmental survival. The *K. pneumoniae* T2SS is composed of 15 different structural protein components that function together to secrete one known enzyme called pullulanase. Previous studies on the contribution of each T2SS component to pullulanase secretion were performed in a heterologous, plasmid-based system in *Escherichia coli* using the *Klebsiella oxytoca* T2SS. Specifically, it was found that proteins PulH and PulM were partially dispensable for pullulanase secretion, and PulB and PulN were fully dispensable. However, it has been shown that the phenotypes observed in this system were impacted by the plasmid copy number, raising questions about the contribution of each structural protein to pullulanase secretion. To clarify the contribution of each T2SS structural component to secretion in *K. pneumoniae*, it is necessary to perform these functional assays directly in *K. pneumoniae*. I hypothesize that all 15 structural components are essential for T2SS function in *K. pneumoniae*. To investigate the contribution of each T2SS structural component, I systematically deleted each of the 15 T2SS encoding genes directly in the *K. pneumoniae* chromosome and complemented each gene in *cis*. Analysis of pullulanase secretion in each deletion and complement strain through an agar-based growth assay revealed unique growth patterns. Deletions in *pulB* and *pulN* do not appear to affect pullulanase secretion while deletions in *pulH* and *pulM* impact secretion to different levels. Furthermore, double deletions of non-essential structural components revealed that Δ*pulH*Δ*pulN* and Δ*pulM*Δ*pulN* show weaker secretion than their individual deletions, indicating potential additive effects. Future work will focus on investigating secretion efficiency through time course enzymatic assays and by using a bacterial two hybrid system to identify specific protein-protein interactions.

Gen abstract type

Poster

Section Type

MGCM

276 - Investigating the Biological Role of the *yobO* Gene in Wall Teichoic Acid Remodelling in *Bacillus subtilis*.

Travis Mammoliti[1], Cullen Myers[1]

[1]University of Guelph

Bacterial cells are encapsulated by a cell wall that is essential for their viability. Its major structural component is peptidoglycan; a continuous macromolecule composed of glycan chains cross-linked by short peptides. Peptidoglycan provides structural rigidity to the cell wall, while governing processes such as cell morphogenesis and division through tight coordination of its assembly with remodelling and degradation schemes. In Gram-positive bacteria such as *Bacillus subtilis*, the cell wall also contains the anionic glycopolymer wall teichoic acid (WTA) as a major component, which plays key roles in diverse cellular processes including cell wall synthesis and homeostasis, morphogenesis, and tolerance to environmental stress. Here, we investigate the biological role of *yobO* in *B. subtilis*, which we have discovered encodes a wall teichoic acid hydrolase. *yobO* is predicted to be subject to catabolic repression, and deletion of *yobO* resulted in impaired growth kinetics under glucose-limited growth conditions. Phase-contrast microscopy revealed that this growth phenotype was accompanied by morphological defects, and the D*yobO* strain exhibited altered cell wall properties that impacted susceptibility to antimicrobials, indicative of an impact to the tolerance of cell envelope stress. The findings support the hypothesis that *yobO* is involved in the tolerance of carbon stress during the transition of *B. subtilis* from vegetative to stationary growth, and position *yobO* as a novel regulator of wall teichoic acid, providing novel insight into the dynamic regulation of WTA in cell wall maintenance and bacterial survival strategies.

Gen abstract type

Poster

Section Type

MGCM

279 - Mitochondrial Dynamics and Population Genetics in Human Pathogenic *Plasmodium* Species

Veronica Thorn[1]

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Malaria remains a pressing global health threat, claiming hundreds of thousands of lives annually. As a eukaryotic pathogen with a complicated lifecycle, *Plasmodium* have been difficult to target with antimicrobials and has high rates of drug resistance. Mitochondria have long been a common target for antimalarial therapies, owing to their substantial structural and functional differences from their mammalian counterpart. These differences, combined with the organelle's indispensability across all stages of the *Plasmodium* lifecycle, make it both a compelling therapeutic target and a uniquely challenging subject of study. To better characterize mitochondrial genetic diversity across the four primary human pathogenic *Plasmodium* species (*P. falciparum, P. vivax, P. ovale, P. malariae*), we used publicly available whole-genome sequence data from the National Center for Biotechnology Information (NCBI) to conduct mitochondrial single nucleotide polymorphism (SNP) population genetics analyses. So far, our analysis has focused on *P. falciparum* and *P. vivax*, including 2,468 and 1,230 isolates, respectively (*P. ovale* and *P. malariae* are included in ongoing analyses). Interestingly, despite the *Plasmodium* cell harbouring only a single mitochondrion, we detected evidence of heteroplasmy of mitochondrial DNA, challenging conventional assumptions about mitochondrial uniformity in these unicellular parasites. *P. falciparum* exhibited notably elevated heteroplasmy relative to *P. vivax*, alongside a greater number of bi-allelic SNPs (97 vs. 62), a higher proportion of pairwise variants failing the four-gamete test (0.6151 vs. 0.1645), and greater linkage equilibrium among SNP pairs, collectively suggesting more active mitochondrial recombination. *P. vivax,* however, had a higher average mitochondrial-to-nuclear ratio than *P. falciparum* (7.6 vs. 3.6), suggesting a greater relative amount of mitochondrial DNA in that species. Geographically structured mitochondrial haplotypes and mitochondrial-to-nuclear ratio variation are predicted across sampled populations. Furthermore, antimalarial-resistant isolates are hypothesized to exhibit elevated mitochondrial-to-nuclear ratio and heteroplasmy levels, suggesting these may represent mechanisms underlying natural drug resistance. Together, these findings continue to shed light on the complexity of apicomplexan mitochondrial biology, with direct relevance to antimalarial drug development, identification of resistance determinants, and a broader understanding of the global population dynamics of this important human pathogen.

Gen abstract type

Poster

Section Type

MGCM

285 - Understanding the role of surfactant molecules in *Streptomyces venezuelae* exploration

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Streptomyces are soil-dwelling actinobacteria characterized by filamentous growth patterns and a complex, multicellular life cycle in which colonies transition from a metabolically active, hydrophilic vegetative mycelium to hydrophobic aerial hyphae and, ultimately, spores (1). This developmental shift from vegetative mycelium to aerial hyphae depends on surfactant proteins—including SapB, the chaplins, and the rodlins—which reduce surface tension and assemble into a hydrophobic sheath that enables hyphae to extend into the air (2,3). In the case of the chaplins and rodlins, this assembly process results in the formation of functional amyloid fibres on the aerial cell surfaces that are organized into an intricate basketwork-like configuration. In addition to this classical life cycle, recent studies have shown that certain *Streptomyces* species can adopt an alternative growth mode termed 'exploration', characterized by rapid lateral colony expansion across solid surfaces that resembles surfactant-mediated sliding motility. Exploring colonies ultimately adopt distinctive wrinkling morphologies that differ depending on the growth medium (4). While the roles of surfactant proteins are well established during classical development, their contributions to exploratory growth remain poorly understood.

To address this, this study aims to investigate the contributions of the three groups of known surfactant proteins (chaplins, rodlins, and SapB) to exploration in *Streptomyces venezuelae*. Targeted gene deletions were generated through PCR-based mutagenesis, and mutant strains were assessed for their ability to undergo exploratory growth across multiple exploration-inducing media. Colony expansion, colony morphology, and competitive fitness were assessed for wild type and mutant strains, to evaluate the structural and functional contributions of these surfactant proteins.

Exploratory phenotypes varied substantially depending on both genotype and growth conditions. The most pronounced differences between strains were observed during co-culture with yeast, whereas glycerol-supplemented conditions promoted robust growth with minimal phenotypic variation. Across all media types tested, chaplin gene deletion resulted in consistent defects in exploration-associated colony expansion, and classical growth-associated sporulation, indicating that the chaplins contribute to exploratory growth, in additional to classical development. In particular, deleting *chpCH* produced more severe growth defects than deleting other chaplin genes, suggesting a functional hierarchy within the chaplin family, with *chpCH* likely encoding the primary polymerization unit that drives aerial hyphae growth and surfactant assembly. In contrast, deleting the rodlins and SapB had minimal effects on overall colony expansion, although loss of the rodlins was consistently associated with altered colony morphology and the emergence of rapidly expanding escaper regions.

Overall, these findings are providing insight into the molecular mechanisms underlying exploratory growth and how functional amyloids and surfactants contribute to *Streptomyces* development. The chaplins appear to be the primary drivers of exploration-associated expansion, while the rodlins and SapB may play more context-dependent or supporting roles.

Gen abstract type Poster

Section Type MGCM

294 - Evaluating the Effect of Carbapenemase Kinetics on the Performance of the CARBA-NP Assay

Aditi Bhaskar[1], Mitchell Jeffs[1], Christopher Lohans[1]

[1]Queen's University

Carbapenemase-producing organisms (CPOs) pose a critical threat to antibiotic therapy because carbapenems are last-line agents for many multidrug-resistant infections. Phenotypic assays like the modified carbapenem inactivation method (mCIM) and the CARBA-NP test are commonly used for rapid carbapenemase detection. These tests offer efficient and accessible diagnostics but exhibit variable sensitivity, particularly for weaker carbapenemases such as OXA-48-like variants. However, these weaker carbapenemases can still confer CPOs with resistance to carbapenems. Therefore, the prompt and accurate detection of weaker carbapenemases like OXA-48 is crucial to improve health outcomes and exercise antibiotic stewardship.

This study focuses on how carbapenemase enzyme kinetics can influence their detection by phenotypic assays. The hydrolysis rate of imipenem, a carbapenem antibiotic, was examined by UV-Vis spectrophotometry and compared against rate of colour change from a kinetic version of the CARBA NP assay. Laboratory strains and clinical isolates producing the five major carbapenemase families (IMP, VIM, KPC, NDM and OXA-48-like) were tested, assessing the relationship between enzymatic activity and CARBA-NP assay performance. These strains were also tested with conventional CARBA-NP assays using established protocols to evaluate the detection of CPOs.

UV-Vis spectrophotometry demonstrated rapid imipenem hydrolysis by KPC-2 and NDM-1 laboratory strains, with slower rates observed for OXA-48 variants. Similarly, the kinetic CARBA-NP test showed rapid colour change for KPC-2 and NDM-1, but slower responses for OXA-48. These findings were supported by the standard CARBA-NP, which detected KPC-2 and NDM-1 but not the OXA-48 variants. Clinical isolates producing KPC, NDM, VIM, and IMI exhibited high rates of colour change with variable levels of imipenem hydrolysis, and all were detected by the standard CARBA-NP. In contrast, OXA-48-like isolates showed variable hydrolysis and colour change rates. Isolates with slower activity were often not detected, while some OXA-48-like carbapenemases displayed varied imipenem hydrolysis rates relative to colour change and tested positive with the standard CARBA-NP.

These results indicate that there is a quantifiable threshold for the detection of weaker carbapenemases by the CARBA-NP. Quantifying this threshold of detection will better inform CARBA-NP detection limits and aid the accurate detection of weaker CPOs. Ongoing work will evaluate a broader panel of carbapenemase-producing clinical isolates. Rates of hydrolysis of different carbapenems, such as meropenem, will also be conducted to evaluate whether trends differ among different carbapenems. Collectively, these findings provide critical insight into refining phenotypic detection via the CARBA-NP assay, ultimately supporting earlier identification of CPOs that produce weaker carbapenemases, reducing the risk of undetected transmission.

Gen abstract type Poster

Section Type MGCM

297 - The human pathogenic *Cryptococcus* Mutation spectrum variation

Megan Hitchcock[1], Jianping Xu[1]

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Mutations are the fundamental source of all variation that enables evolution by providing raw genetic material which natural selection and genetic drift can act. Mutations can arise spontaneously through imperfect DNA replication and repair or through exposure to environmental mutagens. While most mutations are neutral or deleterious, beneficial mutations enable adaptation to changing environments and together, mutations collectively shape genome evolution and species divergence. The mutation spectrum, referring to the rate and type of mutations occurring across a genome has been shown to vary within and among populations. Such variation likely reflects both genetic differences in DNA replication and repair fidelity and the influence of historical environmental exposures. Characterizing these patterns can provide insight into the molecular mechanisms governing mutagenesis and the interaction between genetic and environmental factors that shape genome evolution. The human pathogenic *Cryptococcus* (HPC), a diverse group of basidiomycetous yeasts provides an exceptional model for investigating mutation-spectrum variation in a eukaryotic context. This complex comprises seven closely related species with substantial genetic diversity, enabling comparative analyses across both interspecific and intraspecific populations. As well, these yeasts persist across diverse ecological niches, including soil, trees, avian guano, and mammalian hosts exposing them to heterogeneous environmental conditions that may influence mutational processes. Furthermore, HPC is classified by the World Health Organization as critical priority fungal pathogens, highlighting the need to understand how mutational processes shape their evolution and adaptation. In this study, publicly available whole-genome sequencing data from the National Center for Biotechnology Information are used to investigate patterns of mutation-spectrum variation across four closely related HPC species: *Cryptococcus tetragattii*, *Cryptococcus bacillisporus*, *Cryptococcus gattii*, and *Cryptococcus deuterogattii*. The dataset comprises 580 globally distributed strains, including both clinical and environmental isolates collected over the past four decades. Comparing single nucleotide polymorphs within species to fixed mutations across five selected outgroup isolates, this study aims to infer the ancestral state of mutations to classify mutational type and quantify their frequencies. We will test for significant differences in mutation-type frequencies among species and assess whether variation is structured within or among populations defined by mating type, geographic origin, or ecological niche. Additionally, genome-wide analyses will be conducted to evaluate the spatial distribution of mutation-spectrum variation, enabling the identification of mutational hotspots and their associated genomic functions. We expect that mutation spectra will differ systematically among populations. This study represents the first comprehensive analysis of mutation-spectrum variation across HPC. By identifying species-specific and environment-associated mutational signatures, it aims to elucidate how genetic mechanisms and environmental factors shape the genome of fungal pathogens. These findings will provide a broader framework for interpreting genomic variation in eukaryotic microbes and may contribute to the identification of species-specific determinants of virulence and transmission, with potential implications for diagnostics and disease management.

Gen abstract type Poster **Section Type** MGCM

300 - Biochemical and Biological Analyses of Conserved N-terminal Residues of FtsI and Their Role in Cell Division and β-lactam Susceptibility

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Penicillin-binding protein 2 (PBP2/FtsI$_{Ng}$) of *Neisseria gonorrhoeae* is an essential cell division protein and the primary target for β-lactam antibiotics. FtsI is a transpeptidase involved in cell wall synthesis across bacterial species through its interaction with cell division protein FtsW. While the C-terminal domain of this protein is well-studied because of its penicillin-binding active site, the N-terminal domain remains largely uncharacterized. Bioinformatic analysis has identified 4 amino acids (R75, R167, G180, and E193) in the N-terminus of FtsI from *N. gonorrhoeae* that are conserved across >2000 bacterial species.

We **hypothesize** that these conserved N-terminal residues are integral for the structure and function of FtsI. The **objectives** of this research are to: **1)** characterize the biophysical impacts of mutations to FtsI$_{Ng}$ N-terminus; **2)** observe the influence of the mutations on penicillin-binding; **3)** assess the interaction between FtsI$_{Ng}$ mutants and FtsW$_{Ng}$; and **4)** determine whether FtsI from *E. coli* (FtsI$_{Ec}$) carrying mutations in the conserved amino acids complement an FtsI$_{Ec}$-depleted strain.

Single substitutions in truncated FtsI$_{Ng}$ (FtsIt$_{Ng}$: transmembrane residues 1-48 deleted) were generated by site-directed mutagenesis. To determine whether multiple mutations had additive effects on penicillin binding, constructs of FtsIt$_{Ng}$ combining R75G and R167A or R75A, R167A, G180R, and R75G substitutions were synthesized. Mutant and wildtype His-tagged FtsIt$_{Ng}$ were expressed in *E. coli* and purified by Ni-affinity chromatography. Dynamic Light Scattering (DLS), Multi-angle Light Scattering (MALS), and Circular Dichroism (CD) spectroscopy were used to examine fold and dispersity of the protein. Isothermal Titration Calorimetry (ITC) was performed to measure binding affinities of penicillin to wildtype and mutant FtsIt$_{Ng}$. Bacterial Two-Hybrid (B2H) assays were used to detect interactions between FtsI$_{Ng}$ mutants and FtsW$_{Ng}$ by assessing β-galactosidase activity. The impact of FtsI$_{Ec}$ N-terminal mutations on the survival of *E. coli* was investigated using a conditional FtsI$_{Ec}$-knockout *E. coli* strain expressing FtsI$_{Ec}$ mutants from a low-copy vector.

DLS and MALS indicated that both wildtype and mutant FtsIt$_{Ng}$ are monomeric. CD analysis revealed that single substitutions of the conserved residues have no impact on the secondary structure of FtsIt$_{Ng}$. ITC experiments showed that recombinant FtsIt$_{Ng}$ mutated at R75A/G, R167A/G and G180R increased penicillin binding between ~2- and ~4-fold, as compared to wildtype. Despite having significantly altered secondary folding, determined by CD, FtsIt$_{Ng}$ with multiple mutations exhibited a ~4-fold increase in penicillin binding, similar to that of FtsIt$_{Ng}$ with the R75G mutation. B2H assays demonstrated that single substitution mutations to the conserved residues of FtsI$_{Ng}$ inhibit the interaction between FtsI$_{Ng}$ and FtsW$_{Ng}$. *E. coli* depleted of FtsI$_{Ec}$ could not be complemented with FtsI$_{Ec}$ carrying an E193A mutation in contrast to the other single substitutions in FtsI$_{Ec}$.

These experiments show that the conserved residues in the N-terminus of FtsI$_{Ng}$ are critical sites which modulate both the interactions of FtsI$_{Ng}$ and FtsW$_{Ng}$ as well as penicillin-binding. In mutating these residues of FtsI$_{Ec}$ in *E. coli*, we determined that the E193A mutation impacts the survival of *E. coli*. This study provides insight into the molecular mechanisms of FtsI with implications in understanding antibiotic resistance and cell division processes across species.

Gen abstract type Poster

Section Type MGCM

301 - Prioritizing novel growth inhibitors for antibiotic discovery through chemical-genetic interaction profiling

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The pace of antibiotic discovery has been dramatically overtaken by the development of multidrug resistance. Streamlining the antibiotic development pipeline has profound potential to address this challenge and reshape the treatment landscape for multidrug-resistant bacterial infections. Chemical-genetic interaction profiling (CGIP), which links bioactive compounds to functionally related genes, provides a powerful approach to uncovering new bacterial growth inhibitors and characterizing their mechanisms of action. Previously, our lab developed a pooled library of 612 *Burkholderia cenocepacia* CRISPRi essential gene knockdown mutants. This library was used to screen 5,000 novel small molecules for growth inhibitory activity during competitive growth. The identification of compound-specific decreases in mutant competitive fitness generated a dataset of 69,972 unique chemical-genetic interactions. In this work, a computational pipeline was developed to filter and rank novel growth inhibitors and predicted target proteins from these CGIPs. To this end, 596 putatively essential *B. cenocepacia* proteins were assessed through the lenses of metabolic context, protein-protein interaction network centrality, and druggability. Bioinformatic and statistical approaches were then used to filter growth-inhibitory compounds based on promiscuity, priority of putative target(s), and ADMET similarity to a set of reference antibiotics. In total, 58 high-priority compounds were selected for follow-up. To validate the CGIPs of these compounds in a secondary screening that mimics pooled-strain competition, a dual-fluorescence competition assay is in development. CRISPR-interference essential gene knockdown mutants expressing different fluorescent proteins will be competed against a control strain. Leveraging the distinct excitation and emission spectra of the fluorescent proteins, relative abundance of competing strains will be quantified via fluorescence intensity and used to calculate competitive fitness. By comparing mutants' competitive fitness in the absence or presence of growth inhibitors, compound-specific decreases in competitive fitness can be identified as evidence of specific chemical-genetic interactions. Overall, this research aims to support the antibiotic discovery pipeline by providing a streamlined methodology for discovering, prioritizing, and guiding research into novel bacterial growth inhibitors.

Gen abstract type

Poster

Section Type

MGCM

305 - Characterizing Novel *Salmonella* Hybrid AB5 toxins

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Salmonella is a major cause of foodborne and diarrheal illness, with an estimated 200 million to 1 billion global cases annually. Diarrheal disease is one of the leading causes of death in children under the age of 5 and according to the World Health Organization (WHO) and *Salmonella* is one of the four primary causes of it. In Canada alone, there are an estimated 88,000 cases of salmonellosis annually, resulting in over 900 hospitalizations.

Despite the wide array of virulence factors employed to maintain and establish infections, toxin production is not typically regarded as a feature of salmonellosis. However, a recent discovery may change this. Our lab uncovered two novel putative AB5 toxins within the *Salmonella* genome. AB5 toxins are a common bacterial virulence factor implicated in notable diseases like cholera and whooping cough. They are made up of five B (binding) subunits arranged in a pentameric ring, which confer target specificity, and a single active enzymatic A subunit. Based on genomic sequence analysis it was hypothesized that the proposed toxins were ribosome-inactivating proteins(RIP). RIP toxins work by cleaving a key amino acid residue from the 60S ribosomal subunit of target cells, resulting in apoptotic and necrotic cell death. Currently, the only other known AB5 toxin that shares this RIP enzymatic activity is shiga toxin, an incredibly potent toxin expressed by *Shigella dysenteriae* and Shiga toxin-producing *E. coli* (STEC). Further analysis of the two toxins revealed that they encode different B subunits, each belonging to a distinct AB5 toxin family which uniquely designates them as structural hybrids. One of these toxins contains a pertussis-family B subunit (RIP-TT) and the other, a cholera-family B subunit (RIP-HLT). Of the two toxins, RIP-TT was found to be much more highly conserved among *Salmonella* serovars, consistently displaying over 90% amino acid sequence identity in the 103 screened strains. Based on this, we hypothesize that RIP-TT plays a significant role in *Salmonella* pathogenesis that has yet to be elucidated. By further characterizing these toxins, as well as their potency and secretion patterns we can investigate their role in *Salmonella* infections.

Gen abstract type

Poster

Section Type

MGCM

310 - Improving Genetic Tools for the Genus Burkholderia Through Expanding the Use of Rhamnose-Inducible Promoter

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Genetic engineering tools have enabled precise modulation of gene expression to link bacterial genes to their functions. Rhamnose-inducible CRISPR-Cas systems are widely used in *Burkholderia* species for their tight, tunable control over gene expression. These systems are adapted from *Escherichia coli*, where the rhamnose regulatory system is well characterized. However, the mechanism of rhamnose uptake in *Burkholderia* species remains undiscovered. As a result, when rhamnose-inducible systems fail in certain *Burkholderia,* it is unclear whether the failure is due to ineffective rhamnose uptake.

This project aims to identify the rhamnose uptake system in *Burkholderia cenocepacia* K56-2. Twenty-three candidate loci potentially involved in rhamnose uptake were selected based on whole-genome sequencing of evolved CRISPR interference mutants that had lost their knocked-down phenotype due to impaired dCas9 expression from the rhamnose-inducible promoter. Six of these loci encode a porin protein, an efflux RND transporter and ABC transporters. To investigate if these genetic elements are related to rhamnose transport, we are generating targeted gene deletions for each candidate locus, followed by phenotypic characterization with a plasmid carrying an *eGFP* gene under a rhamnose-inducible promoter. Fluorescence intensity will be measured as a proxy for rhamnose uptake.

A deletion mutant of the porin gene was assessed for GFP expression under rhamnose induction using fluorescence quantification and fluorescence microscopy. Both, wild type and mutant strain carrying the eGFP plasmid showed green fluorescence, confirming that the porin gene was not related to rhamnose uptake. Nevertheless, we demonstrated our pipeline can be used to rapidly identify the involvement of other candidate genes in rhamnose uptake. Construction of other mutants harboring the rhamnose-inducible eGFP plasmid reporter is ongoing and their ability to uptake rhamnose will be presented.

These findings provide the first insights into rhamnose transport in *B. cenocepacia* and establish a foundation for optimizing rhamnose-inducible CRISPR-Cas systems across the genus *Burkholderia.*

Gen abstract type

Poster

Section Type

MGCM

313 - Distinguishing Molecular Patterns in Different Canadian Clubroot Pathotypes

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Clubroot, caused by the obligate parasite *Plasmodiophora brassicae,* is one of the most devastating soilborne diseases affecting *Brassicaceae* crops worldwide. With estimated yield losses ranging from 10% to 100%, the disease continues to threaten global food security. In the major canola-growing regions of Canada, numerous pathotypes have emerged, complicating clubroot mitigation efforts. Several strategies have been implemented to manage this disease, including cropping practices, crop rotations and soil microorganism management. However, breeding for host resistance remains the most effective approach. Over the past decade, the deployment of clubroot-resistant canola cultivars has also been accompanied by the emergence of new pathotypes. This highlights the need for new approaches to better understand the mechanisms underlying the ongoing evolution of *P. brassicae*.

In this study, we used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to profile the proteome of *P. brassicae* resting spores from single-spore isolates representing pathotypes prevalent in Alberta. The single spore isolates were produced and propagated on the universally susceptible *Brassica rapa* cultivar, and galls were harvested six weeks post-inoculation. Proteins were then extracted and analyzed by LC-MS/MS using an Uhrig lab-developed ultra-deep proteome acquisition methodology originally developed for highly complex plant samples. Our findings revealed differences in proteins involved in “carbohydrate metabolic processes” and having “helicase” activity, with some proteins uniquely more abundant in certain pathotypes. Using this approach, we aim to further advance the current understanding of *P. brassicae* virulence. In turn, this work provides critical insights into pathogen evolution that may accelerate the development of new clubroot-resistant cultivars.

Gen abstract type

Poster

Section Type

MGCM

315 - Optimization of a dual-technique approach to assessing *Neisseria gonorrhoeae* biofilm phenotypes

Joyce Kuipers[1], Cezar Khursigara[1]

[1]University of Guelph

Biofilm formation by *Neisseria gonorrhoeae* is an understudied aspect of natural pathogenesis. As the causative agent of the sexually transmitted infection gonorrhea, biofilms are theorized to contribute to asymptomatic infections. These silent infections are highly prevalent in the global population and thus warrant further study to because it is poorly understood why a large portion of individuals remain symptomless. How biofilms may contribute to the virulence of this organism has the potential to inform our current understanding of gonorrhea dissemination and pathogenesis. Thus, it is important to study biofilm phenotypes, the profile of this unique bacterial lifestyle. A current limitation in research is the lack of standard, high-throughput methodologies to study *N. gonorrhoeae* biofilms. The goal of this research therefore is to address this issue by exploring the use of a simple dual-technique approach. Crystal violet assays were combined with resazurin assays to describe both the total biofilm biomass as well as the overall metabolic activity. *N. gonorrhoeae* FA1090 was grown as biofilms to the timepoints of 24, 48, and 72 hours. To assess biomass, a standard crystal violet assay protocol was used. To assess metabolic activity, resazurin at varying concentrations was used to assess an optimal concentration range for semi-quantitative description. The results of this study demonstrated that, over time, the total biomass of biofilms peaked at 48 hours and remained unchanged to 72 hours. The corresponding resazurin assay demonstrated that, between these timepoints, there was a significant reduction in metabolic activity when resazurin was used at a concentration between 0.125-0.25 mg/mL. To assess the utility of this dual approach for evaluating phenotypic changes, biofilms grown to 48 and 72 hours were exposed to 0.6 mg of human hemoglobin in the 24 hours prior to analysis. The result of this screening assay showed a decrease in biomass with a corresponding increase in metabolic activity. This is suggestive of a potential dispersal phenotype not previously described for *N. gonorrhoeae.* Combining crystal violet assays with resazurin assays provides a greater depth of information when describing the phenotypic changes of *N. gonorrhoeae* biofilms in response to environmental changes. This dual assay approach describes unique phenotypic characteristics that traditional crystal violet assays cannot capture alone. Resazurin is an attractive option due to being cost-effective and simple to use. By obtaining both biomass and metabolic activity, this approach could be used to describe different phenotypes of *N. gonorrhoeae* biofilms, with a wide variety of applications in STI research from novel therapeutic molecule screening to describing unique dispersal phenotypes.

Gen abstract type

Poster

Section Type

MGCM

321 - Assessing the role of phage-encoded antibacterial toxins in the lysis-lysogeny decision

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Vancomycin-resistant Enterococci (VRE) are a major cause of hospital-acquired infections, particularly in immunocompromised patients. Many of these bacteria are lysogens, and their integrated phages elements often encode genes which increase bacterial fitness during infection. Despite the impact of lysogenization in determining the bacterial hosts' pathogenicity profile, how phages commit to integration and the gene products that play a role in the phage's virulence life cycle are poorly understood. In the polylysogen VRE, *Enterococcus faecalis* V583, a novel polymorphic toxin family fused to an N-terminal MuF domain, referred to as MuF-associated toxins (Mat), has been identified but remains uncharacterized. Although preliminary data suggests full-length Mat proteins contribute to efficient phage infection, the role of the the N-terminal MuF and the diverse C-terminal toxin domains is not known. However, sequence analysis of Mat prevalence in phage genomes suggest that this family is enriched in phages that preferentially undergo lysogeny. This observation raises the possibility that Mat-mediated antibacterial activity influences whether a phage enters the lytic or lysogenic cycle. Using the V583-infecting phage pp3 as a model system, we explore this further by assessing the phage's ability to infect after generating targeted mutations in the pp3 Mat protein that alter or abolish its antibacterial activity. We will then compare viral particle production, infection efficiency, and lysogeny frequency between wild-type and Mat-mutant phages. These experiments will determine whether Mat activity promotes lysogenic establishment or influences infection dynamics more broadly. By defining the role of Mat proteins in phage life cycle decisions, this project will provide mechanistic insight into how VRE-infecting phages regulate pathogenicity profile in a bacterial host.

Gen abstract type

Poster

Section Type

MGCM

323 - Identification and characterization of novel *Klebsiella pneumoniae* type II secreted proteins

Hannah Braun[1], Thibault Chaze[2], Sylvain Brisse[2], Mariette Matondo[2], Olivera Francetic[2], Jenny-Lee Thomassin[1]

[1]University of Saskatchewan, [2]Institut Pasteur

Klebsiella pneumoniae is a major cause of nosocomial infections and is increasingly difficult to treat due to emerging antimicrobial resistance (AMR). As such, *K. pneumoniae* has gained notoriety as a part of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) which have high rates of AMR in both clinical and community settings. Despite high rates of AMR and *K. pneumoniae* causing a myriad of different infections, the virulence determinants of this bacterium remain understudied. One potential avenue for research is the type II secretion system (T2SS) of *K. pneumoniae.* The T2SS is a nanomachine common in Gram-negative bacteria which secretes folded proteins, called exoproteins, out of the cell into the extracellular milieu. Exoproteins tend to have important roles in bacterial colonization, infection, and interbacterial competition. *K. pneumoniae* possesses a highly conserved T2SS and currently, the only reported exoprotein of the *K. pneumoniae* T2SS is an iso-amylase called PulA. However, since the discovery of PulA, no proteomic analyses have been performed to identify additional exoproteins. In this study, we used a combination of genetic, proteomic and *in vitro* assays to identify and characterize novel exoproteins secreted by the *K. pneumoniae* T2SS. Total secreted proteins from *K. pneumoniae* containing a functional and non-functional T2SS were identified by quantitative mass spectrometry. Mass spectrometry analyses revealed that *K. pneumoniae* strains with and without functional T2SSs have distinct proteomes. Nearly 100 proteins were identified as potential exoproteins. Each potential exoprotein was fused to a C-terminal FLAG tag and Western blot was used to assess secretion in *K. pneumoniae* containing a functional or non-functional T2SS. To date, we have assessed secretion of ~25% of the proteins identified by mass spectrometry and identified 16 new exoproteins. A select subset of these exoproteins have begun to have their functions characterized through *in vitro* enzymatic assays. This study is the first to identify additional *K. pneumoniae* exoproteins. Future work will focus on continuing to characterize the activity and function of newly identified exoproteins and determining how they contribute to *Klebsiella* biology. Identification of novel exoproteins may provide insight into how *Klebsiella* interact with their surrounding environment.

Gen abstract type

Poster

Section Type

MGCM

327 - Balancing Genome Stability and Adaptation: ImuABC-Dependent DNA Damage Tolerance in *Pseudomonas aeruginosa*

Dana Sowa[1]

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Translesion DNA Synthesis (TLS) is a conserved DNA damage-tolerance mechanism that enables DNA replication to proceed across lesions that would otherwise stall replicative polymerases. In some bacteria, TLS is mediated by the ImuA/ImuB/ImuC mutasome, a specialized protein complex implicated in stress-induced mutagenesis and adaptive evolution. While this pathway has been characterized in several species, its functional role in *Pseudomonas aeruginosa* is not well understood.

In this study, we investigated the contribution of ImuA and ImuB to bacterial fitness and DNA damage tolerance using a genetic approach. Deletion mutants of ImuA and ImuB were constructed in *P. aeruginosa*, and their growth phenotypes were assessed under standard conditions in Luria Bertani (LB) media and in stress-inducing conditions in 10% LB media. While there was no growth phenotype observed under standard conditions, the ImuA and ImuB deletion strains exhibited impaired growth relative to wild-type under stress-inducing conditions, supporting a role for the mutasome in maintaining replication efficiency and cellular viability. Complementation of each mutant with its corresponding gene recapitulated growth to near wild-type levels, confirming that the observed defects were specifically attributed to the loss of ImuA and ImuB function.

To further probe genetic interactions and compensatory mechanisms, we isolated spontaneous suppressor mutants from the ImuA deletion background. Notably, these suppressor strains restored growth phenotypes comparable to wild-type despite the continued absence of the respective ImuA gene. This finding suggests that alternative pathways or secondary mutations can bypass the requirement for canonical TLS components.

Together, our results demonstrate that the ImuABC mutasome contributes significantly to growth and replication robustness in P. aeruginosa, likely by facilitating lesion bypass during endogenous or stress-induced DNA damage. However, the emergence of suppressor mutants capable of recapitulating wild-type growth in the absence of ImuA proteins reveals that this pathway is not strictly essential and can be functionally compensated. These findings provide new insight into the redundancy and adaptability of bacterial DNA damage tolerance mechanisms.

Understanding how *P. aeruginosa* balances genome stability with mutagenesis has important implications for pathogenesis and antibiotic resistance, as TLS pathways are often linked to the generation of genetic diversity under stress. Future work will focus on identifying the genetic basis of the suppressor mutants found in this study and investigate the molecular pathways that enable compensation for the ImuA and ImuB loss. In addition, investigating these strains susceptibility to DNA damaging clastogens and antibiotics will help us to further define how the ImuABC mutasome contributes to lesion bypass, replication fork stability, and the coordination of DNA repair pathways under genotoxic stress in *P. aeruginosa*, which can identify this pathway as a novel target in the fight against antimicrobial resistance.

Gen abstract type

Poster

Section Type

MGCM

328 - Study of genetic interactions for antibiotic targets discovery

Siraba Dieynaba Gueye[1], Jordan Mailloux[1], Jean-Philippe Côté[1], Sebastian Portilla[1]

[1]Université de Sherbrooke

In 2019, antibiotic resistance was already responsible for more than one million deaths worldwide, and the number will continue to increase rapidly in coming years. It represents a growing public health problem. The Enterobacteriacea family, counting the well-studied *Escherichia coli*, comprises the primary bacteria responsible for these infections. Antibiotics used to treat them generally target specific functions essentials for the growth of the bacterium, such as DNA replication or peptidoglycan synthesis. However, bacteria can escape antibiotic treatments through antibiotic resistance mechanisms and resistance is on the rise. It is thus urgent to discover new antibiotics with novel mechanism of action. Antibiotic discovery strategies have traditionally targeted the essential genes of a microorganism, which typically represent a small fraction of its genome. Gene essentiality is however dependant in the environmental and genetic context, where the essentiality of a gene will vary according to other genes present in the genome. In extreme cases, synthetic lethality occurs when the simultaneous deletion of two otherwise dispensable genes is lethal. Genes involved in those synthetic lethal interactions are potential novel antimicrobial targets and could provide insight on the implication of those genes in metabolic pathways Here, we validate synthetic lethal interactions that we previously identified using high-throughput interaction studies using a CRISPRi system targeting a gene of interest and a genome-wide mutant library. Using this approach, we validate an interaction between *murA* and *lpxC* in *E. coli* and observed that inhibitors of both enzymes act in synergy. Confirmed synthetic lethal interactions can be used as probes on chemical libraries, opening avenues for new treatments based on synergetic activity.

Gen abstract type

Poster

Section Type

MGCM

329 - High-throughput bacterial phenotyping by high-content microscopy

David Kysela[1], Yves Brun[1]

[1]Université de Montréal

Microscopy has a well-developed and proven history for the detailed analysis of bacterial phenotypes. The multi-dimensional data obtained through the combination of transmitted and fluorescence light microscopy provides sensitive discrimination of both genetic and chemical treatment effects. Here, we describe an approach that extends high-content bacterial imaging, employing multiple fluorescent dyes that target specific cellular structures and physiological activities. We have adapted this strategy to parallel and automation-friendly sample preparation and imaging, thereby enabling analysis of hundreds to thousands of samples per day. We demonstrate application of this approach to the analysis of various antibiotic treatments, using a supervised machine learning model trained on the resulting image data to discriminate phenotypes corresponding to specific antibiotic activities. The resulting model identifies antibiotic treatments from images with high accuracy and even identifies “novel” antibiotic activities not used for model training. This screening approach opens a new avenue for sensitive, high-throughput analysis suited to antibacterial discovery and genome-wide genetic studies.

Gen abstract type

Poster

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MGCM

331 - A Novel Antibiotic-Adjuvant Combination against Multidrug-Resistant Enterobacteriaceae

Joshua Steed[1], Georgina Cox[1]

[1]University of Guelph

While multidrug-resistant *Enterobacteriaceae* are considered critical-priority pathogens for new drug discovery efforts, antibiotic development pipelines are insufficient to address this need, necessitating alternative strategies. The use of antibiotic adjuvants, including compounds that inhibit resistance mechanisms, can enhance the activity of antibiotics limited by intrinsic resistance. Bacterial cell wall biosynthesis is a highly exploited antibacterial target; it depends on the endogenous synthesis of amino sugars that form the peptidoglycan backbone in *Enterobacteriaceae.* Glucosamine-6-phosphate synthase (GlmS) is the sole enzyme capable of producing these amino sugars from neutral sugar precursors; however, GlmS inhibitors have failed against *Enterobacteriaceae* because of a conserved small RNA (sRNA)-based regulatory system that rapidly increases GlmS levels through post-transcriptional target overexpression when glucosamine-6-phosphate is depleted. We have identified an antibiotic-adjuvant pair that exhibits synergistic activity against intrinsically resistant pathogenic *Enterobacteriaceae*, in which subinhibitory concentrations of the RNA polymerase inhibitor rifampicin markedly potentiate inhibitors of GlmS. Systematic deletion of select genes in the GlmS regulatory pathway in *E. coli* identified the sRNAs GlmY and GlmZ as key mediators of this synergistic interaction. Further, activation of this pathway by deletion of *rapZ*, which mediates GlmY-GlmZ signaling, abolished synergy entirely. RT-qPCR analysis revealed that the overexpression of *glmS* induced by a GlmS inhibitor alone was suppressed when in combination with rifampicin. When used in isolation, rifampicin selectively reduced the expression of GlmY and GlmZ without altering *glmS* expression, suggesting a unique vulnerability of sRNA regulators to RNA polymerase inhibition. This selective vulnerability reveals rifampicin as an adjuvant that disrupts sRNA-mediated resistance at subinhibitory levels, thereby enhancing GlmS inhibitors through the suppression of target overexpression. Collectively, these findings establish dual GlmS-RNA polymerase inhibitor combinations as a mechanistically novel antibiotic-adjuvant strategy that overcomes intrinsic sRNA-based resistance to GlmS inhibition in *Enterobacteriaceae*.

Gen abstract type

Poster

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MGCM

334 - Adaptation of a mutagenesis system for performing Tn-Seq in *Enterococcus faecalis*

Ewan Pedersen[1], Casey Fowler[1]

[1]Department of Biological Sciences, University of Alberta

Enterococcus is a genus of gram-positive bacteria that is well known for being a common cause of hospital-acquired infections. The success of Enterococci in the hospital environment is attributed to its biological rigidity, in particular resistance to both antibiotics and disinfecting agents. This has caused the frequency of infections by antibiotic resistant Enterococci to rise sharply over time, which is troubling giving their high mortality rates. Combating this requires obtaining a more complete picture of Enterococcal biology, and a most valuable tool in helping understand Enterococcal biology would be the ability to perform Tn-seq, a technique that involves using transposon mutagenesis coupled to next generation DNA sequencing to unbiasedly assay the whole genome for non-essential genes relevant to a particular phenotype. The objective of this project is bifaceted, the first is to convert existing tools that the Fowler lab has developed for performing Tn-seq in *Salmonella* Typhi for use in the Enterococci model organism *E. faecalis*. We plan to do this using standard cloning approaches to fuse previously developed tools by other labs with Fowler lab tools and techniques to achieve a unified tool capable of performing Tn-seq in *E. faecalis*. The second is to conduct the appropriate set up to allow targeted mutagenesis tools developed by others to be used by the Fowler lab for the purposes of studying *E. faecalis*. If successful this project will create systems allowing for both the identification of relevant genes to a particular phenotype using Tn-seq, and their more in-depth study using targeted mutagenesis. Such a Fowler lab *E. faecalis* mutagenesis toolkit that can then be leveraged in future projects to explore the genetic blueprint of any relevant phenotype in *E. faecalis*, the first of which will be to study Enterococcal resistance mechanisms to disinfectants, which is a key element in their ability to cause hospital acquired infections.

Gen abstract type

Poster

Section Type

MGCM

335 - Molecular characterization of non-O157 Shiga-toxin *Escherichia coli* isolated from Western Canadian cattle

Valeria R. Parreira[1], Opeyemi U. Lawal[2], Kim Stanford[3], Carlton L. Gyles[1], Lawrence Goodridge[1]

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Shiga toxin-producing Escherichia coli (STEC) strains of non-O157:H7 serotypes are increasingly recognized as significant contributors to foodborne illness worldwide. Cattle are a well-established reservoir for both O157:H7 and non-O157 STEC implicated in severe disease in humans. In this study, we performed whole-genome sequencing on 31 E. coli strains isolated from bovine fecal samples to investigate how the resistome, virulome and mobilome diversity contribute to the genomic plasticity of non-O157 STEC. These isolates represented six major serogroups, belonging to13 distinct serotypes and 15 sequence types (ST). Shiga toxin 1c was the most frequently identified stx subtype, followed by stx2d. Strains carrying stx genes exhibited slightly higher virulence gene counts compared to stx-negative strains, and 94% of isolates carried at least one plasmid. Antimicrobial resistance-associated genes emrY, bacA, baeS, mdtF, and cpxA were present in all stx-positive strains. These genes contribute to antimicrobial tolerance through mechanisms such as drug efflux (emrY, mdtF), cell envelope maintenance (bacA), and to envelope stress response (baeS, cpxA). Together, they may enhance the ability of these strains to survive in the presence of various antimicrobial compounds. Phylogenetic analysis showed three distinct clades consistent with the distribution of serogroups and STs. Increasing genomic surveillance to include a broader range of cattle-associated E. coli will further elucidate the evolutionary dynamics and virulence potential of non-O157 STEC.

Gen abstract type

Poster

Section Type

MGCM

338 - Gut Microbial Biomarkers and Tryptophan Pathway Disruption in Major Depressive Disorder: A Multi-Omics Study in a Tunisian Cohort

Nour Elhouda Bouhlel[1, 2], Galal Esmail[1], Walid Mottawea[1], Mouna Mrad[3], Nadia Jaidane[2], Sana Azeiez[2], Rym Ghachem[3], Wejdene Mansour[2], Riadh Hammami[1]

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Gut microbiota dysbiosis is a key factor in the pathophysiology of major depressive disorder (MDD), with the gut-brain axis serving as a key mechanistic bridge between microbial activity and neuropsychiatric outcomes. Despite growing global evidence, the gut microbiome in MDD remains uncharacterized in the Tunisian population. This study aimed to identify gut microbial biomarkers associated with MDD and characterize functional metabolic alterations in depressed individuals compared with healthy controls.

We profiled the gut microbiome of 76 MDD patients and 72 controls using 16S rRNA gene sequencing, followed by shotgun metagenomic sequencing and untargeted metabolomics (nano-flow LC-MS/MS) performed on 24 samples for functional and metabolic profiling. 16S rRNA analysis showed significant differences in β-diversity between the two groups (PERMANOVA $p \leq 0.002$). At the phylum level, MDD patients had higher relative abundances of Actinobacteriota, Proteobacteria ($p < 0.01$), and Cyanobacteria ($p < 0.001$). At the family level, Enterobacteriaceae were increased ($p < 0.001$), while Veillonellaceae were higher in controls ($p < 0.01$). Using the ANCOM-BC model, validated by the ROC curve, we identified four taxa: *Peptoniphilus*, *Eisenbergiella*, *Porphyromonas*, and *Fusobacterium*, as potential depression-associated biomarkers. Shotgun sequencing identified *Faecalibacterium SGB15346* as a species-level biomarker negatively associated with depression, highlighting its relevance in the gut-brain axis underlying depression. Beyond compositional shifts, functional pathway analysis revealed disruption of tryptophan biosynthesis in MDD. Two neuroactive tryptophan-derived metabolites, kynurenic acid and indole-3-propionic acid (IPA), were significantly depleted. Reduced kynurenic acid suggests diminished neuroprotection and heightened neuroinflammatory vulnerability, while IPA depletion is consistent with impaired gut barrier integrity and dysregulated gut-brain signaling. Disruption of this pathway may further contribute to downstream reductions in serotonin and melatonin, potentially underlying the sleep disturbances and mood dysregulation characteristic of MDD.

These findings demonstrate that MDD is associated not only with shifts in microbial composition but, critically, with functional metabolic alterations. Depletion of key tryptophan pathway metabolites positions the gut-brain metabolic axis as a promising target for microbiome-informed diagnostic and therapeutic strategies.

Gen abstract type Poster

Section Type MGCM

339 - Activating the Cpx Response is Required to Delete ftsH in *Escherichia coli*

Monica Kucher[1], Randi Guest[1], Sha Tao[2]

[1]MacEwan University, [2]Princeton University

The membrane-bound protease FtsH is essential for viability in Escherichia coli, where it functions in protein quality control and maintenance of membrane homeostasis. FtsH degrades misfolded membrane proteins and regulates key cellular pathways required for growth. Historically, the lethality associated with loss of FtsH has been attributed to dysregulated lipopolysaccharide biosynthesis. Here we show that, in addition to decreasing LPS levels, the Cpx envelope stress response, which manages misfolded inner membrane proteins, must be activated to delete ftsH in E. coli. We provide evidence to suggest that differential expression of many genes upon activation of the Cpx response allows E. coli to survive in the absence of ftsH. These results suggest that, in addition to regulating LPS biosynthesis, degrading misfolded inner membrane proteins is an essential function performed by FtsH.

Gen abstract type

Poster

Section Type

MGCM

343 - The rhodoglue: the Rhodobacterales way of polar adhesion

Sebastien Zappa[1], Cecile Berne[1], Elise Napolitano[1], Yves Brun[1]

[1]Université de Montréal

Bacteria commonly attach to surfaces to form communities called biofilms. Establishing a biofilm requires a transition from a motile to a sessile lifestyle, which typically involves loss of flagella and production of specialized adhesins. In Alphaproteobacteria, irreversible surface attachment occurs via polar adhesins, of which two types are currently known: the holdfast in Caulobacterales and the unipolar polysaccharide (UPP) in Rhizobiales, each encoded by order-conserved genes.

To explore polar adhesion beyond these orders, we studied the photosynthetic model bacterium *Rhodobacter capsulatus* (Rhodobacterales), known to form biofilms by an unknown mechanism. Biofilm assays and microscopy confirmed polar adhesion in this species. Notably, *R. capsulatus* lacks holdfast and UPP biosynthetic genes, indicating it employs a distinct polar adhesin.

To characterize this novel polar adhesin, we screened a random transposon mutant library for impaired adhesion and validated candidates via targeted gene deletions. Four gene clusters stood out, and homology analyses indicated that the *R. capsulatus* polar adhesin involves sulfated polysaccharides and a homolog of an ABC transporter-dependent capsular polysaccharide synthesis/export system. Fluorescent lectin staining further revealed the presence of galactose in its composition. Additionally, this polar adhesin appears to support attachment across a broad range of surface types, with the highest affinity for hydrophobic ones. Collectively, these features distinguish it clearly from both the holdfast and UPP.

This newly identified adhesin, named Rhodoglue, appears unique to Rhodobacterales, and its distinctness from both known polar adhesins suggests that polar adhesion evolved independently multiple times and is more widespread in nature than previously recognized.

Gen abstract type

Poster

Section Type

MGCM

345 - Solving the "Two-Toxin Dilemma" of Typhoidal Salmonellae

Luke Barretto[1], Casey Fowler[1]

[1]University of Alberta

In 2021, the global burden of typhoid fever was estimated at 9.3 million cases. The causative agent of typhoid fever, *Salmonella* Typhi, uses a unique virulence factor to cause disease in humans: the typhoid toxin (TT). Several studies have shown that TT damages human cells and is directly responsible for some of the symptoms of typhoid fever. Thus, TT is thought to play a key role in how *S*. Typhi persists as a human pathogen. As such, TT offers a promising target to treat cases of typhoid fever. However, this unusual toxin assembles as two different variants which bind different human cells and cause different symptoms. Thus, *Salmonella* experiences a dilemma in balancing the expression of its two forms of TT. How *Salmonella* determines which TT to produce is poorly understood but is thought to be a critical aspect of its biology. Recent data show that *Salmonella* appears to sense pyrimidine or purine starvation to produce one toxin over another. We hypothesize that several genes in *Salmonella* are mediating this unknown mechanism. To understand how *Salmonella* balances TT production, this project uses a targeted screening approach wherein nucleotide biosynthesis genes and gene regulatory systems are deleted in reporter strains of *Salmonella*. Further experiments will be done using RNA-seq to capture differentially expressed genes in WT and nucleotide biosynthesis mutants of *Salmonella*. Understanding how *Salmonella* balances TT production will be important in designing treatments which target TT. Ultimately, this project will advance efforts to combat the global burden of typhoid fever.

Gen abstract type

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MGCM

354 - Bacterial Genetic Interaction Network Analysis using CRISPR interference

Jordan Mailloux[1], Miguel Angel Fernandez Mejia[1], Jean-Philippe Côté[1]

[1]Université de Sherbrooke

Background**:**Cellular functions arise from intricate networks of genetic interactions, in which combinations of genes can exhibit functional dependencies. These interactions become apparent when the combined perturbation of two genes produces a fitness effect that deviates from the expected outcome based on their individual perturbations, revealing functional relationships between them. Techniques such as eSGA (Escherichia coli synthetic genetic array) have enabled the construction of genetic interaction networks and improved our understanding of bacterial cell biology. Despite these advances, our knowledge of synthetic genetic interactions remains limited. Existing methods such as eSGA lack versatility, as they cannot effectively probe interactions involving essential genes and are largely restricted to E. coli.

The objective of this project is to leverage a novel strategy, the SGA 2.0, to map previously unexplored genetic interaction networks. SGA 2.0 employs a mobilizable broad-host-range plasmid, pFD152 that carries an inducible CRISPR interference (CRISPRi) system into which customizable guide RNAs can be inserted via molecular cloning. Plasmids containing a guide RNA against a gene of interest are first introduced into a donor strain and subsequently transferred into recipient strains by conjugation. Because this approach uses inducible CRISPRi, it enables the repression of the target genes by modulating their expression levels rather than deleting them, allowing the possibility to target essential genes. These guide RNA containing plasmids are then conjugated into ordered libraries of single-gene mutants, for instance in Escherichia coli, Salmonella Typhimurium, and Pseudomonas aeruginosa.

SGA 2.0 will enable the systematic identification of genetic interaction networks involving essential bacterial genes across species and provide insights into how global cellular metabolism influences these critical processes. In the long term, the interactions uncovered through this approach may reveal new vulnerabilities in bacterial systems and inform the development of innovative antimicrobial strategies.

Gen abstract type

Poster

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MGCM

361 - Investigating the characteristics and diversity of H-NS-like plasmid protein F (HppF)

Megan Zak[1], Andrew Cameron[1]

[1]University of Regina

The presence of plasmid-borne nucleoid associated proteins (NAPs) has been known for many years, but their functional niche within the cell remains relatively unexplored. Several distinct phylogenetic clades of H-NS family proteins were recently identified and have been coined by our research group as H-NS-like plasmid proteins (Hpps). We examined the HppF clade using a bioinformatic approach, assessing both protein sequence diversity and the broader characteristics of HppF-encoding plasmids. More than 20 unique protein variants were identified within this clade with distinct partitioning of characteristics such as conjugation status, bacterial host preference, replicon type and b-lactamase gene presence based on these variants. HppF-encoding plasmids also shared a high co-occurrence of several stress and virulence genes, suggesting this protein could contribute to plasmid-mediated virulence potential.

Gen abstract type

Poster

Section Type

MGCM

367 - Discovery and Characterization of Novel Endolysins for the industrial Methanotroph *Methylomicrobium album* BG8

Steven Rosvold[1], Lisa Stein[1], Dominic Sauvageau[1]

[1]University of Alberta

The conversion of methane into high-value products by methanotrophic biocatalysts offers a sustainable and promising pathway for greenhouse gas mitigation and the development of a circular bioeconomy. However, the economic viability of these platforms is often hampered by inefficient downstream processing, as recovering intracellular products from robust methanotrophic cell walls remains a significant energy bottleneck. This work addresses this challenge by identifying and validating functional endolysins derived from lysogenized prophages within the genome of type I methanotroph *Methylomicrobium album* BG8. Putative prophage sequences within the *M. album* BG8 chromosome were first identified using bioinformatics tools, including PHASTER, to locate viral regions. Candidate endolysins were prioritized based on phylogenetic conservation and domain architecture, followed by high-resolution structural modeling using AlphaFold 2 to predict catalytic residues and substrate-binding motifs.

The selected lytic genes were synthesized for *Escherichia coli* and cloned into expression vectors for experimental validation. While bioinformatics and structural modeling have revealed several high-confidence prophage derived lysis associated enzymes with conserved glycosyl hydrolase and amidase domains, the heterologous expression and verification of the full candidate library are presently ongoing. Preliminary results from initial candidates have confirmed successful protein production via SDS-PAGE and Western Blotting and *E. coli* autolysis upon induction and suggest that these enzymes possess potent functional activity against bacterial cell wall constituents. This internal disruption provides early evidence of the lytic potential of the enzymes. The research aims to establish a foundational toolkit for methanotrophic harvesting strategies. Future efforts will focus on confirming these effects through enzyme purification and the implementation of expression circuits directly within *M. album* BG8 to streamline methane-based biomanufacturing.

Gen abstract type

Poster

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MGCM

369 - The gene encoding the CadA P1B-type ATPase is linked to silver ion resistance in *Pseudomonas aeruginosa*

Matthew Chu[1], Rahgavi Poopalarajah[1], Shirin Afroj[1], Emily Au[1], Andrea Hassinger[1], Caroline Cui[1], Jillian Doyle[1], Joe Harrison[1]

[1]University of Calgary

Antimicrobial silver has long been used in wound dressings. However, silver resistance remains poorly studied among the ESKAPE pathogens. This includes *Pseudomonas aeruginosa*, which is one of the most frequently isolated bacteria from silver-treated wounds. Here, we sought determinants of silver resistance and discovered that simulated wound fluids containing complement-inactivated human serum induce silver resistance in *P. aeruginosa*. Using strand-specific RNA-sequencing of *P. aeruginosa* exposed to $AgNO_3$ in tandem with susceptibility testing of genome-edited mutants, we identified that a primary determinant of serum-dependent silver resistance is the *cadA* gene. This gene encodes a P_{1B}-type ATPase previously linked to cadmium and zinc resistance. We observed *cadA*-dependent silver resistance in laboratory strain PA14 as well as clinical and environmental isolates. Complementation analysis verified the *cadA* genetic linkage. We found that the function of *cadA* in silver resistance is cryptic in standard laboratory media. Using molecular weight fractionation and medium composition analysis, we determined that a component of heat-inactivated human serum >50 kDa is required for expression of the *cadA*-dependent silver resistance phenotype. Taken together, our data show that *P. aeruginosa* is silver resistant in conditions that integrate key attributes of the human wound microenvironment, and that this resistance depends on a gene for a P1B-type ATPase evolutionarily conserved in the *Pseudomonas* genus.

Gen abstract type

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MGCM

372 - SLAM: Because Surface Lipoproteins Can't Just Walk Through Membranes

Tiana Lee[1], Dixon Ng[1], Megha Shah[1], Christine Lai[1], Trevor Moraes[1]

[1]Department of Biochemistry, University of Toronto

Gram-negative bacteria are adaptable organisms that thrive in diverse environments. To do so, they have evolved surface lipoproteins (**SLPs**) to acquire nutrients, evade host immune defences, facilitate adhesion to target cells, and break down macromolecules through enzymatic activities. These proteins are produced in the cytoplasm and must reach the bacterial cell surface to function; consequently, bacteria have evolved many different secretion systems to shuttle these proteins across the periplasmic space and bacterial outer membrane. The type XI secretion system (**T11SS**) is a two-partner system comprised of an integral outer membrane beta-barrel translocon Slam (**S**urface **L**ipoprotein **A**ssembly **M**odulator) and one or several corresponding SLP substrates. Even though many gram-negative bacteria express multiple Slam homologs, the translocated substrates are highly specific to their corresponding Slam. Furthermore, deletions of either Slam or its substrate significantly reduce virulence in the bacterial pathogen *Neisseria meningitidis*, demonstrating the importance of the T11SS as a key virulence factor. While we have a general understanding of how Slam and its substrates function in bacteria, the precise molecular mechanism for substrate recognition and substrate translocation is not fully elucidated. **We are using a protein engineering and structure-guided approach to identify how each functional domain of Slam coordinates specificity and translocation of its substrate.**

Slam has two functional domains, an N-terminal periplasmic domain and a C-terminal beta-barrel domain within the outer membrane. To pinpoint the specific domain of Slam dictating specificity, we generated a library of hybrid Slam proteins by swapping the periplasmic domains between the three Slam homologs found in *Neisseria gonorrhoeae*. We adapted these neisserial Slam homologs and their corresponding substrates in *Escherichia coli* and then proceeded to test these hybrid Slam proteins in the same system. Our initial findings demonstrate that the N-terminal domain (NTD) is critical for translocation and confers specificity, the exception being the Slam3 NTD hybrid translocons, which facilitated translocation of all Slam substrates in varying degrees.

While the Slam translocon can recognize substrates with high specificity, we have yet to decipher the molecular mechanism behind the substrate translocation process. To identify how these two domains facilitate translocation at the molecular level, we are using cryo-electron microscopy (cryo-EM) to obtain high-resolution structures of Slam in different states of the translocation process. Using purified Slam embedded in detergent micelle, we obtained preliminary 2D classes of Slam particles, which allow us to validate the overall architecture and orientation of the two domains of the translocon. These structural states will provide insight into a novel translocation mechanism of key virulence factors in many gram-negative pathogens. This will provide a basis for the development of novel therapeutics used to inhibit T11SS-mediated virulence and pathogenicity.

Section Type MGCM

Poster session & Abstract selected for MGCM Symposium June 19, 2026

377 - Comparing the detection of Porphyromonas gingivalis and its virulence factors in the oral cavity using multiplex real-time quantitative PCR and digital PCR

Gwendolyn Freeze[1], Benjamin Sinclair[1], Juliana Hermiston[1], Mark Rakobowchuk[1], Jonathan Van Hamme[1], Eric Bottos[1]

[1]Thompson Rivers University

Periodontitis, a chronic inflammatory condition, is characterized by a shift in the oral microbiome toward gram-negative and proteolytic taxa, in which *Porphyromonas gingivalis* is considered a keystone pathogen. *P. gingivalis* drives disease progression through a diversity of virulence factors. Gingipains, for example, are cysteine proteases unique to the species, which play a central role in nutrient acquisition, evading the host immune system, and tissue destruction. Considering the clinical significance of *P. gingivalis*, establishing accurate methods for its detection is increasingly important in human health and clinical research. Quantitative real-time PCR (qPCR) is widely used for microbial diagnostics and has become a pivotal molecular tool in targeted DNA quantification. In contrast, digital PCR (dPCR), a method that enables absolute quantification of DNA without the need of a standard curve, has been shown to offer enhanced precision and sensitivity, and has quickly become a gold-standard method in the field. Bacterial detection using these methods is commonly achieved through targeting the highly conserved 16S rRNA gene. However, detection based solely on this marker falls short of capturing functionally important traits of a species, such as its virulence factors. In this study, we developed a multiplex assay to simultaneously detect the 16S rRNA, hmuY, and kgp genes of *P. gingivalis*, and compared assay performance using qPCR and dPCR methods. The kgp gene encodes a lysine-specific gingipain, while the hmuY gene encodes a heme-binding hemophore-like protein. These virulence factors act synergistically to acquire heme from the environment, an essential growth and virulence regulator of *P. gingivalis*. To assess the performance of the multiplex assay on biological samples, DNA and RNA were extracted from subgingival samples of ten healthy participants. DNA samples were first subjected to 16S rRNA gene amplicon sequencing targeting the V4 hypervariable region, which detected *P. gingivalis* in only one participant. Multiplex qPCR and dPCR were then used to quantify *P. gingivalis* targets (16S rRNA, hmuY, and kgp) in gDNA and cDNA from oral samples. While both methods showed high concordance when tested with serially diluted *P. gingivalis* standards, dPCR demonstrated higher frequency of detection and sensitivity in oral samples compared to qPCR. McNemar's test showed that dPCR detected the 16S rRNA and hmuY targets significantly more frequently than qPCR (16S rRNA: 65% vs 30%, $p = 0.02$; hmuY: 40% vs 5%, $p = 0.02$), while the increase in kgp detection was not statistically significant (20% vs 5%, $p = 0.25$). The Wilcoxon matched-pairs signed-rank test further revealed differences in target DNA concentrations between the two methods, with qPCR non-detections assigned a value of zero. Significantly higher DNA concentrations were observed for the 16S rRNA ($p = 0.005$) and hmuY ($p = 0.02$) targets using dPCR, but not for kgp ($p = 0.25$), suggesting enhanced sensitivity of dPCR for two targets. The performance of this multiplex dPCR assay in detecting low-abundance *P. gingivalis* in healthy individuals provides a valuable proof-of-concept for use in future clinical samples and particularly for assessing key virulence genes.

Gen abstract type Poster **Section Type** MGCM

379 - Neuroprotective potential of *Bacteroides finegoldii* UO.H1052 postbiotics on neuronal cells

Dixi Modi[1], Nazila Nazemof[1], Joseph G. S. W. Mariam[1], Galal Esmail[1], Basit Yousuf[1], Riadh Hammami[1]

[1]NuGut Research Platform, School of Nutrition Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada

Major depressive disorder is associated with impaired inhibitory neurotransmission, including dysregulated γ-aminobutyric acid (GABA) signaling, and increased neuroinflammation. Emerging evidence suggests that gut microbiome-derived postbiotics influence neuronal function through bioactive metabolites and extracellular vesicles (EVs); however, their mechanistic roles remain poorly defined. This study investigates the neuroprotective potential of postbiotics derived from the psychobiotic candidate *B. finegoldii* using an *in vitro* neuronal stress model. A physiologically relevant stress model was established using differentiated and undifferentiated SH-SY5Y cells exposed to graded concentrations of cortisol (0.1-100 μM) and tumor necrosis factor-α (TNF-α; 10 μM). Cellular stress responses were evaluated using ATP-based viability, lactate dehydrogenase (LDH) cytotoxicity assays, and morphological analysis. Low-dose cortisol (0.1-0.5 μM) with TNF-α (10 μM) established a physiologically relevant neuronal stress model, supported by LDH assays showing minimal membrane damage. In contrast, cortisol alone induced mild cytotoxicity (~20%). Dose optimization identified *B. finegoldii* cell-free supernatant (2.5-5%) and extracellular vesicles (3.6×10^6 particles/mL) as effective, non-cytotoxic treatments that promote favorable cellular responses. Using this optimized model, postbiotic effects from the wild-type strain and a CRISPR-engineered *gadB*-deficient mutant are being evaluated by RNA sequencing to elucidate GABA-dependent mechanisms of neuronal stress resilience and neuroprotection. This work provides mechanistic insight and supports the development of *B. finegoldii* as a next-generation psychobiotic candidate for stress-related neurological disorders.

Gen abstract type

Poster

Section Type

MGCM

383 - Shikimic acid as a modulator of microbial dynamics and biofilm establishment in Listeria species

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[1]Cell and systems biology department, University of Toronto

Listeria persistence in food-processing environments remains a major food safety concern because its ability to form biofilms which makes contamination difficult to control and increases the risk of recurrent outbreaks. This is particularly relevant in plant-based food preparation systems, where metabolites such as shikimic acid are naturally abundant and support microbial growth. Although *Listeria* species convert shikimate into protocatechuate through the QuiC2 enzyme, the biological significance of this pathway remains unclear.

This study examined the role of QuiC2 in *Listeria innocua* as a model to understand how shikimate-derived metabolites influence biofilm establishment and microbial interactions. This rationale stems from the observation that *Listeria* produces protocatechuate despite lacking the capacity to use it as a carbon source, suggesting a role in surface colonization or competition within mixed microbial communities. We therefore tested whether QuiC2 affects biofilm development and interspecies dynamics, particularly with *Pseudomonas putida*, a common environmental bacterium.

Using wild-type *L. innocua* and a *quiC2* deletion mutant, we performed growth assays, crystal violet biofilm quantification, microscopy, and hydrophobicity analyses under glucose, shikimate, and combined glucose-shikimate conditions. These experiments were conducted in co-culture with GFP-labelled *P. putida*, enabling species differentiation based on fluorescence.

The wild-type strain produced protocatechuate and exhibited enhanced biofilm formation, increased matrix protein content, and greater surface hydrophobicity under shikimate-supplemented conditions. In contrast, the *ΔquiC2* strain formed weaker biofilms, showed reduced hydrophobicity, and was less competitive in co-culture, with *P. putida* dominating under these conditions. Structural analyses showed that wild-type biofilms were more heterogeneous and enriched in proteinaceous extracellular matrix compared to those produced with the mutant.

Together, these findings suggest that protocatechuate produced via QuiC2 contributes to biofilm formation, adhesion, and interspecies competitiveness in *Listeria*. This identifies QuiC2 as a modulator of biofilm-associated traits and supports a role for shikimate-derived metabolites in shaping microbial behavior. These results highlight a potential target for controlling *Listeria* biofilms and provide insight into how natural plant metabolites may influence microbial persistence.

Gen abstract type

Poster

Section Type

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387 - Redundant or Strategic? Insights into *Streptomyces* Isolates Harboring Clavam- and Carbapenem-Type β-Lactam Biosynthetic Gene Clusters

Kajal Gupta[1], Arshad Shaikh[1], Nader Abusara[1], Madelyn Swackhamer[1], Kapil Tahlan[1]

[1]Memorial University of Newfoundland

β-Lactam antibiotics and β-lactamase inhibitors are critical tools in addressing antimicrobial resistance, yet their natural diversity and co-occurrence remain incompletely understood. Clavulanic acid (CA), a well-known β-lactamase inhibitor, is typically co-produced with cephamycin C in *Streptomyces clavuligerus*, with their biosynthetic gene clusters (BGCs) located adjacently. However, CA-like BGCs are also found in other bacteria without associated cephamycin C clusters. In this study, we investigated *Streptomyces pratensis* and ten environmental *Streptomyces* isolates harboring CA-like and MM 4550-like carbapenem BGCs, but lacking cephamycin C BGCs. Although these strains do not produce CA, they synthesize predicted monocyclic β-lactam intermediates that may represent ancestral β-lactamase inhibitors. Several isolates exhibited both antimicrobial and β-lactamase inhibitory activities, indicating active carbapenem biosynthesis. Gene disruption analysis confirmed that MM 4550-like BGCs contribute to both antibiotic and inhibitory functions, whereas CA-like clavam BGCs are limited to β-lactamase inhibition. These findings reveal a broader natural distribution and co-occurrence of β-lactam BGCs than previously recognized, suggesting functional interplay and evolutionary significance. Importantly, this work identifies previously underexplored sources of β-lactamase inhibitors and antibiotics, highlighting their potential for discovering novel therapeutic combinations to combat antimicrobial resistance.

Gen abstract type

Poster

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MGCM

395 - Characterizing Cytotoxicity and Host Pathway Interactions in Mycobacteriophage Charlie

Adam Al-Khalili[1], Rebecca Santhosh Babu[1], Hosein Abardeh[1], Tracy Xie[1], Gil Toex[1], Elizabeth Williams[1], Adam Rudner[1]

[1]University of Ottawa

As the silent pandemic of antibiotic resistance continues to grow, it is imperative to explore alternative therapies such as the use of bacteriophages to combat infections. Bacteriophages, or phages, are viruses that infect and kill bacteria, and are the most abundant biological entities on Earth, with an estimated population of 10^{31}. Despite this abundance, bacteriophage biology is poorly understood, as over 70% of phage genes have no known function. The objective of this project is to investigate the molecular mechanisms by which the mycobacteriophage Charlie interacts with its host bacterium, *Mycobacterium smegmatis.* This study investigates the functional effects of Charlie genes on host growth to identify molecular mechanisms needed for viral infection.

We have conducted a genome-wide screen on all 68 of Charlie's genes to determine which genes cause toxicity when expressed in the host. All of Charlie's genes were cloned into an anhydrotetracycline (aTc) inducible plasmid and overexpressed in *M. smegmatis*, to assess their impact on host growth. 15 genes cause varying levels of cytotoxicity, indicating potential disruption of essential host processes. Three genes are extremely cytotoxic and fail to yield transformants in *M. smegmatis* after electroporation. Introduction of a premature stop codon after the start site allowed transformation of these genes, suggesting that leaky expression of these genes causes their cytotoxic phenotype.

We have now begun to examine the host interactions of select Charlie genes and the host proteome using both co-immunoprecipitation of tagged genes and an *E. coli*-based bacterial two-hybrid screen. Efforts to tag genes with mCherry have allowed us to assess the expression of five Charlie genes after aTc induction but have revealed that the fused mCherry protein is cleaved either while being expressed in *M. smegmatis*, or within extracts of these cells. This cleavage limits the usefulness of this tag in co-immunoprecipitation studies. We are now testing whether adding a 3X_FLAG tag can be used to monitor expression of phage genes, and successfully immunoprecipitate protein complexes.

Overall, characterizing interactions between phage proteins and their bacterial hosts is critical for elucidating the molecular mechanisms that phages use during infection. Defining the functions of previously uncharacterized genes will help address key gaps in our understanding of phage biology.

Gen abstract type

Poster

Section Type

MGCM

400 - Genetic analysis of Cpx regulated IM protein, YfgG for stabilization of the cytochrome bo3 complex under oxidative stress.

Vera Hanne Ong[1], Ethan Shaigec[1], Tracy Raivio[1]

[1]University of Alberta

The Cpx envelope stress response is a two-component system thought to respond to a wide variety of stressors associated with envelope perturbations. Recent studies have demonstrated that the Cpx envelope stress response plays a role in coordinating macromolecular complexes within the inner membrane, including respiratory proteins. The electron transport chain is a crucial component of aerobic respiration, and in Gram-negative bacteria, these proteins along with other essential metabolic proteins, are embedded within the inner membrane. Under stressful conditions, the Cpx system has been shown to directly repress transcription of the *nuo* and *cyo* operons.

This study aims to understand the genetic regulation of *yfgG* expression by the Cpx envelope stress response and its interaction with members of the respiratory chain. We report that under Cpx activation, *yfgG,* which encodes for a small inner membrane protein is upregulated. YfgG confers resistance to aerobic stressors, Cobalt (II) and Nickel. Luminescence analysis revealed that *yfgG* expression it is more significant in conditions where Cpx is active. Similarly, Δ*yfgG* strains demonstrated increased sensitivity to cobalt(II) and nickel in metal sensitivity assays, supporting its role in alleviating aerobic stress. Moreover, growth curve analysis revealed that overexpression of YfgG increases survival in the presence of cobalt (II) in both wildtype and Δ*yfgG* backgrounds.

Under conditions that affect aerobic respiration, the Cpx pathway upregulates YfgG to stabilize the cytochrome bo3 complex of the aerobic respiratory chain. Genetic analysis of cobalt (II) resistant suppressor mutants in a Δ*yfgG* background revealed mutations cyoA, suggesting potential interaction between YfgG and the cytochrome bo3 complex. Affinity purification of 6XHis-tagged YfgG in cobalt (II) treated cells demonstrated co-purification with four other proteins that were identified by mass spectrometry to be components of cytochrome bo3. Similarly, co-immunoprecipitation also supported this finding, indicating a physical interaction between YfgG and cytochrome bo3.

Structural predictions generated using AlphaFold 3 support a direct interaction between YfgG and the CyoB subunit. In addition, luminescence reporter analysis of YfgG showed upregulation under conditions where CyoB is overexpressed, consistent with interaction. Furthermore, crosslinking studies confirmed this interaction.

Collectively, our findings support a model in which the Cpx envelope stress response upregulates YfgG under conditions that disrupt aerobic respiration through the stabilization of inner membrane respiratory complexes, particularly the cytochrome bo3 terminal oxidase.

Gen abstract type Poster

Section Type MGCM

403 - Genome Annotation a Lytic Phage Infecting *Bacillus altitudunis* BD6 Isolated from a Dye-polluted site

Imade Nsa[1], Ayodeji Odunsi[2], Peter Abiona[1]

[1]University of Lagos, [2]Alabama A&M University, USA

The study of bacteriophages has helped to expand current knowledge on evolution and diversity of microbes. *Bacillus altitudinis* BD6 isolated from a dye polluted site in Lagos, Nigeria has decolorizing capabilities. The genome sequence and gene calls of *Bacillus* phage ALT-BI5 were extracted from *B. altitudunis* BD6 using PHASTEST (PHAge Search Tool with Enhanced Sequence Translation) software and webserver v.3.0 and manual curation was done using DNA Master v.5.23.6. Its genome size is 43,361bp and it has a GC content of 51.66%. So far the sixteen putative genes that have been identified in phiALT-BI5 have functions in replication (DNA primase, DNA polymerase, DNA Helicase, HNH endonuclease), assembly (head-tail connector protein, scaffolding protein) and structural organization (major head protein, tail fibre, tail tube). The detection of an endolysin, holin and spanin suggest a lytic life cycle. The genes that have been identified are in the forward orientation. PHAGE_Bacillus_ALT-BI5 is most closely related to PHAGE_Entero_phiKDA1_NC_027980 sharing the following putative genes: Rz-like spanin, endolysin, DNA primase, virion proteins, DNA maturase A, DNA maturase B, DNA-dependent RNA polymerase, DNA polymerase, DNA helicase, Head-to-tail connector, HNH Endonuclease VII, 5'-3' Exonuclease, capsid protein, tail tubular protein A and tail protein B, tail fibre, scaffolding protein and several hypothetical proteins. Lastly, PHAGE_Bacillus_ALT-BI5 can be predicted to be of the podovirus morphotype because of its resemblance to PHAGE_Entero_phiKDA1_NC_027980. The examination of this uncultivated phage did not reveal the presence of phage genes associated with lysogeny or bacterial genes related to dye decolorization.

Gen abstract type Poster

Section Type MGCM

413 - A covalent adhesin mediates predator-prey attachment and highlights new mycobacterial biology

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Predatory bacteria have attracted growing interest as tools for studying and targeting bacterial pathogens. Motivated by this, we investigated the recently described epibiotic predator of the Mycobacteriales, *Candidatus* Mycosynbacter amalyticus, a member of the Candidate Phyla Radiation. The mechanisms of predation in this lineage remain almost entirely unknown. Even so, predation requires adhesion to prey that is both specific and durable. Here we identify a new adhesin, ThioP, that mediates the covalent attachment of *M. amalyticus* to its prey. Using a recombinant ThioP-mCherry fusion and fluorescence microscopy, we showed that the adhesin forms localised foci on target cells, recognising sites of active cell wall biogenesis. A series of in vitro adhesion assays revealed that ThioP binds covalently to the prey's peptidoglycan, a mechanism supported by a 1.5 Å crystal structure of its adhesin domain. Considered alongside the microscopy, these data provide the first evidence that mycobacterial peptidoglycan is solvent-exposed at discrete foci. ThioP is conserved throughout the phylum, suggesting that a covalent bridge between predator and prey peptidoglycan is a conserved predatory strategy, which we confirmed in the distantly related *Nanosynbacter lyticus*. Beyond illuminating predation in these understudied bacteria, ThioP offers a useful tool for probing peptidoglycan structure and dynamics.

Gen abstract type

Poster

Section Type

MGCM

APPLIED & ENVIRONMENTAL MICROBIOLOGY (AEM) ABSTRACTS/ RÉSUMÉS DE MICROBIOLOGIE APPLIQUÉE ET ENVIRONNEMENTALE (AEM)

1 - Potent Antioxidants from Radioresistant *Kocuria* sp. TMM-11: Repressing Photoinduced Oxidative Damage in Edible Plants

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Ultraviolet (UV) radiation is a major abiotic stress factor that adversely affects plant growth, productivity, and physiological performance by inducing oxidative damage. In recent years, extremophiles have gained attention as a promising source of bioactive metabolites capable of mitigating stress-induced damages in plants. However, the potential of extremolytes from UV-resistant bacteria to protect edible plants from photo-induced stress remains to be investigated. The present study aimed to isolate and characterize intracellular pigmented metabolites from the UV-resistant *Kocuria* sp. TMM-11, assessing their potential as natural UV-stress protectants in wheat (*Triticum aestivum* L.). *Kocuria* sp. TMM-11 was isolated from Thal Desert (Pakistan) soil samples. Based on phylogenetic analysis, it shares the greatest similarity with *Kocuria sediminis* and *Kocuria turfanensis*. Resistance to UV radiation was verified using survivability assays conducted under both UV exposure and oxidative stress. The pigmented metabolites (also termed as extremolytes) were solvent-extracted and subsequently purified using silica gel column chromatography followed by solid-phase extraction. The purified fraction demonstrated strong antioxidant and iron chelation activity, significantly inhibiting oxidative damage to proteins and lipids while preventing DNA strand breaks *in vitro*. Phytotoxicity assessment indicated that the metabolic extracts of *Kocuria* sp. TMM 11 were non-toxic to plants, with seed germination rates observed above 80%. The UV/Vis spectrum revealed three characteristic absorption peaks (λmax) between 250 and 450 nm, indicating the presence of carotenoids in the extract. Key carotenoid compounds detected in LC-MS analysis confirmed the identification of rhodovibrin, phytoene, 4'-Hydroxy-4,4' diaponeurosporene-4-oic acid and 3,4,3',4'-Tetrahydrospirilloxanthin. The plant-level application involved three stages: biopriming of wheat seeds, foliar application of varying extremolyte concentrations, and a subsequent controlled exposure to UV stress. Key metrics measured included chlorophyll content, oxidative stress markers [malondialdehyde (MDA) and proline], and antioxidant enzyme activities. Subjected to UV stress, the plant demonstrated significant reductions in biomass and chlorophyll, concurrent with a marked elevation in oxidative stress markers. The foliar application of extremolytes (2 mg/mL) significantly enhanced plant growth, improved physiological parameters, and increased chlorophyll content, while reducing levels of MDA and proline. The extremolytes boosted the activity of key antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX), which contributed to the mitigation of cellular damage caused by reactive oxygen species. These findings indicate that the extremolytes from *Kocuria* sp. TMM-11 effectively alleviate UV-induced oxidative damage and enhance stress tolerance in wheat.

Overall, this study demonstrates the potential of microbial-derived pigmented metabolites as eco-friendly bioprotectant to enhance plant resilience against UV stress. These findings provide a scientific basis for developing sustainable agricultural strategies that utilize extremophile-derived bioactive compounds to mitigate abiotic stress and improve crop performance under challenging environmental conditions.

Gen abstract type Poster

Section Type AEM

3 - From PET to PHA: Bioconversion of Plastic Monomers by a Novel *Glutamicibacter mishrai* Strain

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Polyethylene terephthalate (PET) poses a serious threat to ecosystems, wildlife, and human health. In contrast, polyhydroxyalkanoates (PHAs) are biodegradable polymers synthesized by microorganisms and offer a promising alternative to conventional plastics. This study aimed to produce PHA using newly isolated bacterial strains capable of metabolizing PET monomers such as terephthalic acid (TPA) and ethylene glycol (EG) as carbon sources. Soil and PET residue samples were collected from the PET recycling industry. PHA-producing strains were screened using Nile Blue A and Sudan Black B staining. Positive isolates showed bright orange fluorescence and black granules, respectively. Bacterial strain PR31, showed the highest performance, was selected and characterized through morphological, biochemical, and molecular methods. Based on 16S rRNA gene sequencing, strain PR31 was identified as *Glutamicibacter meshrai*, reported here for the first time for PHA production using TPA and EG as a carbon source. Physicochemical parameters including temperature, pH, inoculum size, incubation time, and nutritional factors were optimized using statistical tool, Plackett-Burman and Central Composite Designs. *Glutamicibacter meshrai* PR31 exhibited optimal growth at 30 °C, pH 7.0, and 72 h of incubation. Maximum biomass yields were 3.13 g/L and 2.71 g/L, with corresponding PHA yields of 3.93% and 2.85%, using 24 mM TPA and 30 mM EG, respectively. The extracted PHA was characterized by GC-MS and identified as medium-chain-length polyhydroxybutyrate (PHB). This study demonstrates the efficient utilization of PET monomers as carbon sources for microbial PHA production by a novel bacterial strain, contributing to sustainable bioplastic development.

Gen abstract type

Poster

Section Type

AEM

4 - Microbial Community Restructuring and Functional Responses in PFOA Enrichment Cultures: Insights from Molecular Profiling and Strain-Level Characterization

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Per- and polyfluoroalkyl substances (PFASs) are highly persistent environmental contaminants that exert sustained selective pressure on microbiomes[1]. Although microbial tolerance to PFAS has been increasingly reported[2], it remains unclear whether PFAS-impacted communities retain their structural integrity and functional responsiveness following long-term preservation and renewed selective exposure. Within the framework of the SUS-MIRRI.IT project, this study addresses a critical challenge in microbial resource management: preserving environmentally relevant microbiomes while maintaining their adaptive potential. Soil and sediment samples collected from PFAS-impacted sites were cryopreserved at −80 °C using 30% (v/w) glycerol for up to 12 months. Following preservation, samples were subjected to stepwise enrichment cultures using perfluorooctanoic acid (PFOA) (300 mg L^{-1} to 600 mg L^{-1}) to impose progressive selective pressure. Microbial communities were analyzed at four time points: T0 (pre-storage), T1 (3 months), T2 (8 months), and T3 (12 months). A combination of molecular and cultivation-dependent approaches was employed to evaluate community structure, genotypic diversity, and functional response. DNA extraction indicated consistent biomass recovery across all storage durations, with sediment-derived enrichments yielding higher DNA concentrations than soil. PCR-DGGE profiling of 16S rRNA gene fragments revealed the persistence of several dominant bands over time, suggesting that a substantial portion of the original microbiome remained stable under PFAS selection. However, shifts in band intensity and diversity indicated progressive restructuring of dominant populations from T0 to T3. Cluster analysis demonstrated matrix-dependent dynamics, with sediment communities showing relatively higher similarity across time points compared to soil. Similarity between T0 and T3 decreased to approximately 20% in sediment and 5% in soil. Despite these compositional changes, microbial proliferation was sustained under enrichment conditions. Total counts reached up to 10^9 CFU mL^{-1} in soil and 10^8 CFU mL^{-1} in sediment for bacteria, while fungal populations increased to 10^7-10^8 CFU mL^{-1}. These findings indicate that a large fraction of the preserved microbiome remained viable and capable of growth under PFAS-induced stress. To evaluate functional potential, enrichment-derived new microbiomes were subjected to 87-day batch experiments containing PFOA (25 mg L^{-1}) and PFHxS (10 mg L^{-1}). Across all treatments (abiotic control, Soil T0, Sed T0, Soil T3, and Sed T3), PFAS concentrations remained comparable to initial levels. No evidence of degradation/transformation was detected, highlighting the limited biodegradation potential under the tested aerobic conditions. Microbial isolation yielded 138 bacterial and 53 fungal strains, with BOX-PCR fingerprinting resolving 103 distinct bacterial operational taxonomic units, reflecting high genotypic diversity. Partial 16S rRNA gene sequencing revealed temporal enrichment of genera such as *Pseudomonas*, *Microbacterium*, *Stenotrophomonas*, *Raoultella*, and *Rhodanobacter*. ITS sequencing identified fungal taxa including *Candida*, *Trichosporon*, *Galactomyces*, *Geotrichum*, *Cyberlindnera*, and *Fusarium*. These taxa represent stress-tolerant and metabolically versatile groups adapted to PFAS-impacted environments. Overall, PFOA enrichment imposed strong selection pressure, leading to the restructuring of distinct microbial communities in both soil and sediment matrices. While microbial persistence and adaptability were evident, no corresponding PFAS removal was observed. This study highlights the need to decouple microbial persistence from functional PFAS-transformation potential and emphasizes the importance of integrative structural-functional frameworks in evaluating microbiome-based bioremediation strategies.

Gen abstract type Poster

Section Type AEM

6 - Not every gene is special: one simple rule to control the false discovery rate when analysing high-throughput sequencing data

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Differential expression and differential abundance analyses are commonplace in studies employing high-throughput sequencing approaches; however different tools often fail to return comparable results when applied to the same dataset. Most tools employ various normalisations to attempt to correct for technical variation in the count data. We have previously demonstrated that these normalisations are often inappropriate due to incorrect assumptions regarding the overall scale (i.e. size) of the biological system in question. In this study, we conducted 100 permutation analyses of 10 different RNA-seq datasets into which we injected a pre-specified amount of read count 'signal' using binomial thinning. For each analysis instance, group membership of the samples was permuted so that all features identified as differentially expressed are false-positives, except for those whose counts were altered by thinning (i.e. true positives). We show that the problem of scale misspecification results in poor control of the false discovery rate by several commonly-used tools for differential expression analysis in RNA-seq experiments. Moreover, we demonstrate that this can be ameliorated by using a scale model in ALDEx2 or ALDEx3 to account for uncertainty around the size of the system scale. We show that there is an inherent trade-off between satisfactory control of false-discovery rates and sensitivity and that no tool offers both. We also quantify how increasing scale uncertainty affects the difference between groups required for a feature to be reported as differentially expressed. Finally, we provide universal guidance on choosing an appropriate amount of scale uncertainty for any type of analysis. Overall, our work highlights the strengths and pitfalls of commonly used tools for differential expression analyses and highlights the choice between sensitivity and false-discovery rate control that all researchers are making when analysing sequencing data.

Gen abstract type

Poster

Section Type

AEM

10 - Mitigating the Zoonotic Risk of Shiga-Toxigenic *Escherichia coli* (STEC) O157:H7 in Cattle: Evaluation of *Limosilactobacillus reuteri* as a Direct-Fed Microbial

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Background: Shiga toxin-producing *Escherichia coli* (STEC), particularly serotype O157:H7, remains a major food safety concern in North America. Cattle are the primary reservoir, shedding STEC in feces and contributing to contamination of beef products and farm environments. Direct-fed microbials (DFMs) are beneficial microorganisms administered to livestock to improve gut health and potentially reduce pathogen load. Among them, *Lactobacillus* spp. are widely used due to their probiotic properties and ability to inhibit enteric pathogens.

Objective: This study evaluated whether *Limosilactobacillus reuteri* R0444 could reduce STEC O157:H7 shedding in calves.

Methods: Two independent animal trials were conducted in 2025. After a two-week adaptation period, twelve Holstein male calves (13-14 weeks old) per trial were randomly assigned to a control group inoculated with a five-strain mixture of nalidixic acid-resistant STEC O157 or a treatment group receiving both STEC and *L. reuteri* R0444. All calves were orally inoculated with 10^{10} CFU of STEC O157 on Day 0, while calves supplemented with *L. reuteri* (10^{10} CFU) on d 0, 10, and 17. Fecal samples were collected on d 1, 3, 5, 7, 10, 15, 22, and 28 to quantify and detect STEC shedding using selective plating and immunomagnetic separation (IMS). The quantity of DFM strain in fece was monitored by qPCR throughout the experiments. Fecal microbiota was characterized by 16S rRNA gene sequencing processed in QIIME2 with DADA2. Data were analyzed using generalized linear mixed models (SAS PROC GLIMMIX).

Results: Across both trials, STEC shedding differed by treatment ($p = 0.04$) and time ($p < 0.001$), with DFM treatment reducing shedding by an average of 0.7 $\log_{10}$ CFU/g of feces. When the study period was stratified (Days 1-3, 5-7, and 10-28), a treatment-by-time interaction was detected ($p = 0.03$) along with a strong effect of time ($p < 0.001$). Differences between groups became evident after the initial post-inoculation phase. Treated calves shed fewer STEC ($p < 0.05$) during Days 5-7 and Days 10-28 compared with controls. The largest difference occurred on Day 22 ($p = 0.003$), when only 1-2 treated calves shed STEC (1-2.5 $\log_{10}$ CFU/g), whereas 3-5 control calves shed higher levels (1.4-5.6 $\log_{10}$ CFU/g). At Day 28, STEC O157 was not detected by IMS in 67% (Trial 1) and 83% (Trial 2) of DFM-treated calves. In contrast, STEC was recovered from 67% (Trial 1) and 100% (Trial 2) of control calves. qPCR detected *L. reuteri* R0444 shortly after administration, but levels declined to below detection at later sampling points.

Conclusions: Administration of *L. reuteri* R0444 reduced STEC O157 shedding in calves, supporting its potential as a DFM-based pre-harvest intervention to mitigate this pathogen in cattle.

Gen abstract type

Poster

Section Type

AEM

18 - Hydrophobic Antimicrobial Coatings for High-Touch Surfaces: A Scalable Strategy for Infection Control

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Together with the growing threat of antibiotic resistance among human pathogens, healthcare-associated infections (HAIs) present a persistent challenge to healthcare systems [1]. In Canada, approximately 8% of hospitalized patients acquire HAIs in addition to the initial reason for admission [2], highlighting the need for improved infection-control solutions alongside the development of novel treatment strategies.

Here, we address the issue of pathogen transfer through frequently touched surfaces, such as door handles or medical device interfaces. These surfaces mediate pathogen transmission [3], while current disinfection strategies rely heavily on human compliance, which has proven to be insufficient [4].

We propose dual-action antimicrobial coatings engineered for durable, long-term pathogen control on frequently touched surfaces. Our approach is based on modification of aluminum to generate nanostructured functional oxide layers, followed by immobilization of antiseptic onto the functionalized surface. The nature of the coating compound, combined with the honeycomb-like nanostructure, contributes to increased surface hydrophobicity, reducing affinity for contaminated aerosols such as mucus or saliva droplets. The antiseptic, in turn, drives the contact-killing properties of the coating. At this stage, pilot coatings were evaluated for antimicrobial activity using an ISO-style droplet-based antimicrobial assay [4], demonstrating over 90% reduction in *Pseudomonas aeruginosa* surface burden after one hour of exposure. Surface hydrophobicity was assessed via water contact angle measurements, with the most hydrophobic variant exhibiting a contact angle of approximately 120°. Scanning electron microscopy was performed to complement these findings.

This study demonstrates progress toward a scalable, cost-effective antimicrobial surface technology that supports a One Health framework by reducing pathogen transmission at the human/animal-environment interface without chemical release. The contact-killing, non-leaching nature of the coating minimizes environmental impact while enabling passive, long-term protection across healthcare and community settings where prevention of surface-mediated bacterial contamination is critical.

Literature cited:

1. Zimlichman, E., Henderson, D., Tamir, O., et al. 2013. Health care-associated infections: A meta-analysis of costs and financial impact on the U.S. health care system. *JAMA Intern. Med.* 173(22): 2039-2046.

2. Mitchell R, Taylor G, Rudnick W, et al. Canadian Nosocomial Infection Surveillance Program. Trends in health care-associated infections in acute care hospitals in Canada: an analysis of repeated point-prevalence surveys. CMAJ 2019;191:E981-8

3. Otter, J.A., Yezli, S., Salkeld, J.A.G., and French, G.L. 2013. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. Am. J. Infect. Control. 41(5 Suppl): S6-S11.

4. ISO 22196. 2011. Measurement of antibacterial activity on plastics and other non-porous surfaces. International Organization for Standardization, Geneva, Switzerland.

Gen abstract type

Poster

Section Type

AEM

27 - Staphylococcal phages with broad activity and milk stability to control mastitis in Kenyan dairy goats

Audrey Addablah[1, 2], Xavier Minne[1, 2], Elodie Fortin-Hurtubise[1, 2], Laurence Vallières[1, 2], Brice Tsafack Metangmo[1, 2], Nicolas Svitek[3], Sylvain Moineau[1, 2]

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Mastitis is one of the most prevalent and costly diseases affecting dairy livestock, in both developed and developing countries. While the disease has been extensively studied in dairy cows, it remains underexplored in goats despite their growing importance in small-scale and subsistence systems. In Kenya, goats are the most abundant livestock and contribute significantly to household income through milk and meat production. Goat farming also has important gender dimension, as women frequently manage the animals and participate in milk commercialization. In this context, mastitis in goats is not only an animal health issue but also a socio-economic challenge, particularly in rural settings. Coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* are the predominant pathogens associated with goat mastitis. Conventional antibiotic-based strategies are limited by restricted access to veterinary services and appropriate antimicrobials, misuse of antibiotics, and the emergence of antimicrobial resistance. There is therefore a need for alternative strategies. Bacteriophages (or phages) represent an option due to their specificity, self-amplification, and ability to suppress bacterial growth. Our objective is to develop a staphylococcal phage cocktail as a proof-of-concept treatment for mastitis in dairy goats.

To identify effective candidates, we screened 33 staphylococcal phages against 85 mastitis-associated isolates from goats and cows. While siphophages and podophages generally exhibited narrow host ranges, a subset of myophages showed broad activity, infecting more than 70% of both *S. aureus* and CoNS isolates. This broad infectivity is consistent with the ability of myophages to bind staphylococcal cells independently of wall teichoic acid glycosylation, enabling cross-lineage activity. Among the tested phages, Stab21 displayed the widest host range (81.16%) and was prioritized for further evaluation.

We then assessed killing efficacy using individual phages and two-phage cocktails centered on Stab21. Against *S. aureus*, single phages produced only transient inhibition followed by bacterial regrowth, suggesting rapid resistance emergence. In contrast, phage cocktails maintained complete growth suppression over 24 hours. For *S. caprae*, both single phages and cocktails achieved complete inhibition. Finally, we evaluated phage stability in raw and pasteurized goat milk. All selected phages retained infectivity after 24 hours. These findings indicate that goat milk provides a favorable matrix for maintaining phage infectivity and support applications in goats.

In conclusion, our study provides a clear proof-of-concept for the use of phages to control caprine mastitis, both *in vitro* and in milk. The results highlight myophages as strong candidates for broad activity across *Staphylococcus* lineages. Functionally, single-phage treatments were prone to resistance, whereas two-phage cocktails maintained complete suppression over 24 h. The complete control of *S. caprae* by individual phages further suggests that simplified formulations may be suitable in CoNS-dominated contexts. The preserved infectivity of the selected phages in raw goat milk reinforces their translational potential and supports their suitability for on-farm applications. Finally, given the limited number of antimicrobials specifically approved for use in lactating dairy goats in Canada, this work may also be valuable in the Canadian context.

Gen abstract type Poster

Section Type AEM

35 - Metabolism of Polyethylene terephthalate by a Novel Bacterial Consortium Produces Valuable By-Products

Mitchell Slobodian[1], Dominique Jillings[2], Aditya Barot[1], Jessica Dougherty[3], Kalpdrum Passi[2], Sujeenthar Tharmalingam[1, 3], Vasu Appanna[1]

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In this study, we report a newly developed bacterial consortium mainly comprised of the novel bacterium *Paraburkholderia fungorum* capable of metabolizing polyethylene terephthalate (PET) and its derivatives as the sole carbon source in a defined mineral medium. Our unique bacterial consortium naturally metabolizes PET and its derivatives to produce valuable by-products, and we have improved the efficiency of PET metabolism through genetic engineering. Our proposed technology is similar the bacterial technology currently used in Northern Ontario mines but applied to a different pollutant. To date, we have confirmed the composition of our consortium through 16S rRNA sequencing and grown our bacterial consortium in liquid media containing terephthalate (TPA), a derivative of PET, as the sole source of carbon. We have confirmed growth of each bacterial species using 16S amplicon metagenomics. Additionally, we have confirmed that our consortium can produce valuable by-products such as p-cresol, catechol, and mandelic acid through liquid chromatography mass spectrometry (LC-MS/MS) and high-performance liquid chromatography (HPLC). We have successfully developed and maintained a bacterial consortium capable of consuming TPA as the sole source of carbon and producing economically valuable products and published our results in the journal Microorganisms in September 2025. The future directions will be to insert the genes for *MHETase* and *PETase* into *P. fungorum*. MHETase and PETase are enzymes first isolated from the bacterium *Ideonella sakaiensis* which can hydrolyze PET to break down the plastic into the monomers TPA and ethylene glycol. The ability of this microbial community to efficiently convert PET into high-value by-products offers a promising and potentially economically sustainable approach to addressing plastic pollution.

Gen abstract type

Poster

Section Type

AEM

42 - Comammox *Nitrospira* and AOA improve nitrification and protect against ammonia and nitrite accumulation in newly established freshwater aquarium biofilters

Alexander Umbach[1], Owen Ikert[1], Benoit Choquet[2], Josh Neufeld[1]

[1]University of Waterloo, [2]Hagen Industries Ltd.

The home aquarium industry is represented by an estimated 9 to 12 million home systems in North America alone, containing hundreds of millions of individual aquatic organisms and generating a global annual revenue of $70 billion CDN. When establishing a new freshwater aquarium, accumulation of harmful ammonia and nitrite is a concern because these ions are toxic to fish even at relatively low concentrations. Although many home aquarists minimize ammonia and nitrite accumulation in newly established aquaria by using conventional supplements (CS) to promote nitrification, research on microbial community composition and *in situ* effectiveness of available supplements is lacking. Because recent literature demonstrates that complete ammonia-oxidizing (comammox) *Nitrospira* and ammonia-oxidizing archaea (AOA) dominate aquarium biofilters, we tested enrichment cultures containing comammox *Nitrospira* (CX) and the ammonia-oxidizing archaeon *Ca.* Nitrosotenuis aquarius (AQ) for use as nitrifying supplements for newly established aquaria, evaluating their efficacy alongside a representative CS culture. The results demonstrate that inoculating new aquaria with nitrifying supplements resulted in unique supplement-associated nitrifier profiles within each treatment group and that these populations contributed to ammonia- and nitrite-oxidizing activity. Maximizing supplement dose was effective at increasing oxidation of ammonia to nitrate. Treating aquaria with CX, either as the sole supplement or in combination with AQ or CS, prevented ammonia and nitrite accumulation, especially when aquaria were treated with the highest tested dose. Treating aquaria with CX also provided persistent protection against nitrite accumulation following acute fish-free ammonia loading of up to 100 µM ammonium chloride. Whereas 16S rRNA gene sequences associated with CS were detected rarely within biofilter profiles, those associated with CX and AQ established early and persisted temporally. Overall, these results highlight potential benefits of using CX as a home aquarium nitrifying supplement and demonstrate how nitrifiers are differentially adapted to freshwater aquarium conditions.

Gen abstract type

Poster

Section Type

AEM

43 - Microbial response to rapid bentonite saturation and compression supports proposed dry density threshold for a deep geological repository

Luke Schofield[1], Katja Engel[1], Rachel Beaver[1], Chang Seok Kim[2], Josh Neufeld[1]

[1]University of Waterloo, [2]Nuclear Waste Management Organization

Deep geologic repositories (DGRs) are the planned safe long-term storage solution for used nuclear fuel worldwide. Designs include the use of multiple engineered barriers, including used fuel containers, bentonite buffer (a swelling clay), and the surrounding host rock. In Canada's design, used fuel containers (UFCs) will be encased in highly compacted bentonite blocks and placed at depths of 650-800 m below ground surface in emplacement rooms excavated from crystalline rock, with spaces between bentonite blocks and the host rock filled with a granular bentonite material (i.e., "gap fill material"). Past research has shown that microorganisms exist in as-received bentonites, some of which could negatively influence the longevity of a DGR, for example through microbially influenced corrosion of the UFCs by sulfate-reducing bacteria (SRB). Previous research has focused on pump-saturated pressure vessels to simulate early stages of groundwater infiltration in a DGR. Results show suppression of microbial growth in compacted bentonite after a relatively slow saturation process is completed and high swelling pressures have been established. To study microbial suppression after post-bentonite saturation, while minimizing potential residual effects of microbial growth during saturation, pressure vessels were used to rapidly compress pre-wetted gap fill material to dry densities of 1.25,1.30, 1.35, 1.40, or 1.45 g/cm^3. This approach targeted 100% full saturation prior to incubation of the saturated bentonite "plugs" for 1 month at room temperature. Following incubation, bentonite plugs were analyzed using cultivation and 16S rRNA gene sequencing. Results revealed decreasing abundances of culturable aerobic heterotrophs with increasing bentonite dry density. Relative to the as-received bentonite starting material, no significant increase in abundance of culturable microorganisms was detected for the highest tested dry density (1.45 g/cm^3; $p < 0.05$). In contrast, all lower dry densities showed significantly greater abundances of culturable aerobic heterotrophs compared to the as-received bentonite. These samples were associated with relatively high abundance of *Bacillus*-associated ASVs that were not detected in 1.45 g/cm^3 samples. Both cultivation and sequencing results demonstrated that a bentonite dry density of 1.45 g/cm^3 suppresses microbial growth post-bentonite saturation, which is the target average dry density for repository gap fill material. This research also presents a method for studying microbial suppression in bentonite, without the influence of slow saturation effects, allowing for rapid testing of post-saturation microbial processes.

Gen abstract type

Poster

Section Type

AEM

44 - Seasonal Succession and Trophic Partitioning in an Arctic Freshwater Microbial Loop

Mukund Ramesh Gauthankar[1, 2, 3], Milla Rautio[1, 2, 3], Catherine Girard[2, 3, 4]

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Arctic freshwater ecosystems undergo extreme seasonal shifts. However, the microbial interactions that maintain carbon balance across these shifting gradients remain poorly understood. We investigated the seasonal partitioning of top-down and bottom-up controls on the prokaryotic community in Greiner Lake, Nunavut. Across an 8-campaign sampling continuum (August 2022-August 2023), epifluorescence microscopy and flow cytometry were utilized alongside high-resolution physical profiling (temperature, conductivity, dissolved oxygen) to infer interactions between phototrophic nanoflagellates (PNF), heterotrophic nanoflagellates (HNF), bacteria, and virus-like particles (VLPs). Linear regression models of community abundance revealed a highly partitioned, "two-speed" microbial ecosystem. The system is fundamentally bottom-up limited; bacterial abundance positively correlated with PNF abundance ($r = 0.57$, $p = 0.0003$), suggesting a primary carbon pump driving resource control. However, inferred top-down regulation was distinctly partitioned by season and extreme physical bottlenecks. During the prolonged darkness of winter ice-cover, the photosynthetic carbon pump crashed. To endure this severe energy deficit, the bacterial community faced simultaneous suppression from persistent viral pressure (Virus-to-Prokaryote Ratios of 15-19) and a dominant HNF population that likely shifted to mixotrophy/heterotrophy. Following spring ice-out, a sudden influx of light and nutrients triggered a massive bacterial expansion, immediately met with a peak in viral abundance ($>2.30 \times 10^7$ VLP/mL). This positive correlation ($r = 0.46$) suggests a density-dependent viral response to the spring bloom. Conversely, during stable late-summer stratification, HNF populations completely decoupled from bacterial standing stock ($r = -0.06$), pointing to steady, density-independent protozoan control. This chronological partitioning suggests that viral and protozoan populations trade top-down dominance to maintain ecosystem stability across dynamic, climate-sensitive Arctic successions.

Gen abstract type

Poster

Section Type

AEM

47 - Microbes as Biosorbents for Indium: Evidence for Intracellular Uptake

Richa Verma[1, 2], Avery Moy[1], Aditya Barot[1, 2], Jessica Dougherty[2], Sujeenthar Tharmalingam[2], Vasu Appanna[1]

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Indium is a critical mineral that is commonly lost to mine tailings and secondary waste streams due to the absence of selective and environmentally compatible recovery methods. While microbial systems have been proposed as biosorbents, current approaches largely assume extracellular ligand-mediated metal binding, and the mechanisms governing indium interaction with microbial systems remain poorly defined.

In this study, a mixed microbial consortium was exposed to indium stress under different carbon sources to evaluate indium uptake and partitioning. Cultures were monitored over a 0-72 h time course for growth, metabolic responses, and community-level changes. Indium distribution between extracellular, cell-associated, and intracellular fractions was quantified using ICP-MS, while extracellular metabolites and microbial composition were assessed using HPLC and 16S rRNA gene sequencing.

The consortium maintained growth under indium exposure and exhibited carbon-source-dependent responses. However, extracellular ligand signatures were not dominant, and indium was primarily localized within the biomass fraction, indicating intracellular sequestration as the major mechanism of indium handling. These findings suggest that microbial indium recovery may be governed by metabolism-driven uptake and community adaptation rather than extracellular ligand production, highlighting an alternative biological pathway for indium recovery.

Gen abstract type

Poster

Section Type

AEM

52 - Dietary and environmental factors override host genetics in shaping bat microbiomes across microbial domains

Chadabhorn Insuk[1], Xingyuan Su[1], Adrian Cam[1], Cori Lausen[2], Jianping Xu[1]

[1]McMaster University, [2]Wildlife Conservation Society of Canada

Bats play a crucial role in North American ecosystems, yet the ecological processes shaping their microbiomes remain poorly understood. Here, we integrate multi-marker DNA metabarcoding approaches to characterize dietary inputs, host-associated microbial communities, and their environmental connections across three common bat species in Western Canada (*Eptesicus fuscus*, *Myotis yumanensis*, *Myotis lucifugus*). By examining both bacterial and fungal assemblages across host body sites and prey, we explore how microbiomes are structured across biological and ecological interfaces.

Our analyses reveal complex but non-uniform connections between host-associated microbiomes, with domain-specific patterns of connectivity across body sites. While fungal communities exhibited significant associations between wing and gut microbiomes, bacterial communities remained strongly compartmentalized. Patterns of overlap between bats and their prey suggest that trophic interactions contribute to microbial assembly, although these relationships vary across taxa and ecological contexts. Notably, host genetic relatedness explains little of the observed microbiome variation, indicating that other ecological factors may play a dominant role.

Together, these findings highlight the dynamic and multi-layered nature of microbiome assembly in wild mammals, emphasizing the importance of environmental and dietary factors in shaping host-associated microbial communities. This work underscores the need to consider microbiomes within a broader ecological framework, with implications for understanding wildlife health and ecosystem function under a One Health perspective.

Gen abstract type

Poster

Section Type

AEM

56 - Bioremediation and Sequestration of Gallium from Mining Tailings using Bacterial Derived Chelators

Amanda Keursten[1], Sujeenthar Tharmalingam[2], Vasu Appanna[1], Mitchel Slobodian[1], Aditya Barrot[1], Richa Verma[1], Jessica Dougherty[1]

[1]Laurentian University, [2]NOSM University

Using *Pseudomonas fluorescens*, we aim to develop a sustainable, cost-effective method to reclaim gallium from mining tailings. Gallium is a critical material for advanced technologies, including semiconductors and solar panels, as well as applications in medical imaging, cancer therapies, and antimicrobial treatments. Despite its importance, gallium extraction is costly and geopolitically constrained. Zinc and aluminum mining, industries in which Canada is prominent, generate tailings containing trace amounts of gallium; however, no widely implemented recovery methods currently exist, and proposed approaches are often inefficient or economically unviable. Recovering gallium from these waste streams offers the potential to secure a domestic supply while addressing environmental concerns associated with mining.

Our proposed approach harnesses siderophores, metal-binding molecules secreted by bacteria, to selectively extract gallium. Although siderophores naturally bind iron, we aim to induce *P. fluorescens* to produce gallium-selective variants through stress-induced evolution. To evaluate the feasibility of this approach, we investigated how bacterial growth and metabolic activity respond to gallium exposure and adaptation in a gluconate-based system.

Adaptation to gallium significantly enhanced bacterial growth under gallium stress conditions, particularly at intermediate concentrations (0.5 mM), where adapted cultures exhibited increased protein production relative to non-adapted controls. At higher gallium concentrations (1 mM), growth was suppressed overall, though adaptation continued to confer a measurable advantage. Changes in pH closely paralleled growth dynamics, with adapted cultures showing greater pH increases under gallium exposure. Strong positive correlations ($r > 0.9$) between growth and pH across most conditions indicate that pH changes reflect metabolic activity. However, this relationship was disrupted in non-adapted cells under high gallium stress, suggesting a decoupling of growth and metabolism that is mitigated through adaptation.

These findings demonstrate that *P. fluorescens* can adapt to gallium and maintain metabolic activity under metal stress, an essential prerequisite for sustained siderophore production in gallium-containing environments such as mining tailings. This adaptive resilience supports the feasibility of biologically mediated gallium recovery strategies. Building on this foundation, we propose a scalable column-based filtration system in which bacterially produced siderophores selectively bind gallium, followed by recovery through acid elution.

This approach offers a dual benefit: enabling sustainable gallium recovery while mitigating environmental impacts of mining waste. More broadly, it highlights the potential of leveraging microbial adaptation and metabolism to address global challenges related to critical mineral supply and resource sustainability.

Gen abstract type Poster

Section Type AEM

57 - Genomic Epidemiology of Clinical Clostridioides difficile Isolates from Patients in Alberta, Canada

Sanjana Kalra[1], Clinton Luk[1], Dylan Pillai[2], Andrew Doxey[1], Abiola Olaitan[1]

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Introduction: *Clostridioides difficile* (*C. difficile*) is an anaerobic, spore-forming, gram-positive bacterium that causes *Clostridioides difficile* infection (CDI), a leading cause of healthcare-associated diarrhea. CDI remains a burden in Canada, with one of the highest incidence rates among high-income countries. Recent studies have shown a shift in the epidemiology of *C. difficile*, including a decline in RT027 and increase in ribotypes such as RT106. In addition, increasing genetic diversity in toxin genes, along with reduced susceptibility to CDI antibiotics has been observed in clinical isolates. Despite these developments, genomic studies of Canadian clinical strains remain limited. Therefore, this study aims to address this gap by investigating the genomic epidemiology of *C. difficile* in Canada.

Methods: A total of 178 stool samples were collected from CDI patients in Calgary, Alberta. Samples were ethanol-treated and cultured on selective CCFA agar for *C. difficile* isolation. Isolates were confirmed by PCR targeting the *hpdBCA* operon. Antibiotic susceptibility profiles were determined using agar dilution minimum inhibitory concentration testing for CDI treatment antibiotics (metronidazole, vancomycin, fidaxomicin and rifaximin) and epidemiological markers (moxifloxacin and tetracycline). DNA was extracted for ribotyping and whole-genome sequencing using Nanopore. Genomes were assembled using Flye and annotated with Prokka. Toxin gene profiles (*tcdA*, *tcdB*, *tcdC*, *tcdE*, *tcdR*, *cdtA*, *cdtB*, and *cdtR*) were identified using Blastp, and toxin subtypes were assigned using Diffbase. Phylogenetic analysis was performed using IQTree to assign a clade to each isolate.

Results: A total of 155 isolates were confirmed as *C. difficile* by PCR and included for further analysis. Antibiotic susceptibility testing indicated that 6% (9/149) of isolates were resistant to metronidazole, 2.8% (4/145) to vancomycin, 2.0% (3/151) to rifaximin (RIF), while none were resistant to fidaxomicin. Among epidemiology markers, 6.3% (9/144) of the isolates were resistant to moxifloxacin and 4.8% (7/145) to tetracycline. Ribotyping revealed RT014-020 (25.8%) as the most prevalent ribotype, followed by RT016 (12.3%) and RT002 (7.1%). Most isolates exhibited an A+B+CDT- toxin profile (71.6%), followed by A+B+CDT+ (11.0%), A-B-CDT- (7.7%), and A-B+CDT+/A-B+CDT- (1.3%). Toxin subtyping identified multiple variants of toxin A (A1-A3) and toxin B (B1, B2, B4, B7, B9) with A1.1 and B1.2 being the most prevalent. Resistance-associated SNP analysis showed A-to-C SNP in P*nimB* (6/9; metronidazole resistance), T115A SNP in *vanR* (3/4, vancomycin resistance), R505K SNP in *rpoB* (2/3; rifaximin resistance) and T82I SNP in *gyrA* (6/9; moxifloxacin resistance). Some resistant isolates have not been analyzed yet.

Discussion: This study highlights the evolving epidemiology of *C. difficile* in Canada, with a predominance of non-hypervirulent but epidemic ribotypes, particularly RT014-020. Antimicrobial resistance was detected in a subset of isolates and was associated with resistance-conferring SNPs, suggesting an emerging shift in susceptibility that may impact treatment outcomes. The predominance of A+B+CDT- toxin profile indicates that most circulating strains aretoxigenic but not hypervirulent, with a smaller subset of CDT-producing lineages. The presence of multiple toxin subtype suggests potential functional differences in toxin activity, although their clinical impact remains unknown. Overall, these findings emphasize the importance of continue genomic surveillance to monitor resistance and virulence patterns.

Gen abstract type Poster

Section Type AEM

60 - The effect of biofilm formation on interbacterial competition in Pseudomonas aeruginosa

Margaret Kitney[1], Sean Booth[1]

[1]University of Manitoba

Bacteria either live as free-swimming individuals or in surface-attached, aggregated clusters called biofilms, which grow within a matrix of self-produced extracellular polymeric substances (EPS). Biofilms are associated with many chronic infections that resist antibiotic treatment. Developing a better understanding of how biofilm formation protects bacteria from stressors could lead to more effective management of problematic biofilms. One important stressor is interbacterial antagonism, as densely packed bacteria within biofilms compete for limited space and resources. Beyond competition for nutrients, bacteria produce a variety of molecular weapon systems to directly inhibit or kill rival bacteria. We used the opportunistic pathogen *Pseudomonas aeruginosa* as a model of biofilm formation and weapon production to determine if biofilms provide protection from different types of molecular weapon systems. *P. aeruginosa* produces various weapons that operate at different ranges, including long-range pyocin S2 and short-range contact-dependent inhibition (CDI). We tested the potency of pyocin S2 and CDI in conditions of enhanced and restricted biofilm formation by altering a necessary aspect of stable biofilms - cellular aggregation. Aggregation was enhanced or restricted by increasing (Δ*wspF*) or decreasing (Δ*cdrA*) expression of the adhesin CdrA. Competitions were conducted between wildtype attacker and pyocin S2 or CDI susceptible strains. Both attacker and susceptible strains had the same aggregation genotype. We found that both short- and long-range weapons were less effective in the Δ*wspF* background, likely due to aggregated bacteria being more difficult for attackers to access. Surprisingly, while pyocin S2 was as or more effective in the Δ*cdrA* background compared to the wildtype, CDI was less effective in Δ*cdrA* strains compared to the wildtype, despite the expected disadvantage of decreased aggregation. Further study is needed to determine why Δ*cdrA* protected bacteria from CDI; this could indicate that CDI relies on transient adhesion mediated by CdrA to attack competitors. Overall, these results suggest that the evolution of biofilm formation may be influenced by interbacterial competition. Studying the effects of aggregation on competition will improve our understanding of biofilm formation and could provide insight into managing persistent biofilm infections.

Gen abstract type

Poster

Section Type

AEM

62 - The Riverine Plastisphere Floating Through Calgary; Impacts on Community Composition and Co-occurrence

Jonas Stadfeld[1]

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Microplastics (plastics < 5 mm) are ubiquitous, polluting the air, soil, and water across ecosystems worldwide. They have been detected in a wide range of organisms and in human tissues. Beyond their physical presence, microplastics interact with microbial life, as they are colonized by microbes, forming distinct surface-associated communities known as the plastisphere. The plastisphere has been highlighted as a potential biotechnical approach to addressing microplastic pollution, as many of its microbial inhabitants have the capacity to degrade microplastic polymers. However, this potential is tempered by a potential enrichment of pathogenicity and antimicrobial resistance in the plastisphere. Thus, this duality of the plastisphere warrants investigation into plastisphere ecology to understand the factors that influence the abundance of these consequential metabolisms. In this study, we aim to evaluate the impact of an urban landscape, as this environment is associated with an increase in microplastic abundance as well as pathogenicity and antimicrobial resistance genes. Hence, we investigated whether freshwater plastisphere dynamics shift along an urban landscape gradient.

We used Nile red staining to isolate microplastics from water and sediment samples collected from the Bow and Elbow Rivers upstream, within, and downstream of Calgary. Subsequently, we assessed microbial communities from microplastic, background water, and background sediment using 16S rRNA gene amplicon sequencing.

We found that both water and sediment plastisphere communities differed from their respective backgrounds and from each other. Interestingly, many taxa were shared between the water plastisphere and the sediment samples, but not the background water. This suggests that background sediment could be uniquely contributing taxa to water plastisphere assemblage in our study area. Therefore, environmental factors that may disrupt or increase the frequency of this interaction, such as waterbody depth and water-column dynamics, may influence freshwater plastisphere community assemblage. Additionally, plastisphere composition and co-occurrence patterns varied between upstream and downstream sites. These differences were driven by many taxa, including Comamonadaceae, Rhodobacteraceae, and *Escherichia*. Some Comamonadaceae and Rhodobacteraceae taxa have been reported as MP-degrading, suggesting that downstream sites may have more MP-degradation potential. Thus, our results indicate that urban landscapes influence the freshwater plastisphere, suggesting that human activity may modulate its ecological and functional impacts.

Gen abstract type

Poster

Section Type

AEM

65 - Developpement of new comparative methods of microbiomes using functional potential

Vincent Primpied[1, 2, 3, 4], Elsa Rousseau[1, 2, 3, 4], Clovis Galiez[5]

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Microbiomes are increasingly recognized as playing an important role on their environment, such as on biogeochemical cycles or on human health. Studying and linking them to environmental traits require the use of metagenomic sequencing and computational approaches. As opposed, wet-lab studies generally only investigate a portion of the communities due to limitations in the cultivation of microorganisms. Among sequencing technologies for the microbiome, whole-metagenome shotgun sequencing is becoming increasingly affordable, high-throughput and accurate, making retrieve whole genomes from the community possible. The investigation can be undertaken by considering the community as a whole and assessing whether a change in an environmental condition leads to a change in the community composition, which involves comparative methodologies of the microbiome.

State-of-the-art comparative methods are based on the genomic similarity between samples. Among those, a set of methods compare taxonomic profiles, the abundance for each present taxa in a sample. Examples of metrics are Jaccard, on presence/absence, Bray-Curtis, on abundances, and Unifrac, adding phylogenetic information.

Yet this set of approaches requires the ability to assign taxa to sequencing reads. However, given the considerable diversity in microorganisms, genomic databases are not exhaustive. This results in a portion of the sequencing data not receiving any assignation. For reference, MetaPhlan 4, the state-of-the-art tool for metagenomic taxonomic assignation, has an estimated fraction of classified reads as low as 4% for soil microbiota. To address that limitation, alternative methods measure the similarity between samples directly from genomic sequences, therefore using all the genetic material. Typically, genomic dissimilarity tools, such as Simka, finds all nucleotide sequences of length k, k-mers, computes either presence/absence or count data of these k-mers in each sample, and then calculate the dissimilarity between each pair of samples using a metric, such as Jaccard distance. These approaches have improved the quality of comparisons by making full use of metagenomic data.

However, this characterization remains fundamentally linked to genomic, and thus evolutionary and geographic proximity. Indeed, two genomically distant microbiota samples may have common functional adaptations due, for example, to similarities in their environments.

Therefore, here we propose two new comparative methods of microbiota samples based on their functional potential: ProClAP (Protein Clusters Abundance Profiles) and OTOPE (Optimal Transport Over Protein Embeddings). To extract function, the tool Prodigal extract the set of proteins encoded in the genomes. Where ProClAP use a Pearson correlation to compare samples, OTOPE embed the data using protein Language Model before computing dissimilarity.

To compare our methods and other widely used metrics, we use Tara Ocean dataset, composed of 180 shotgun metagenomic marine samples from across the globe along with nutrient and environment metadata. Once the different methods applied, ProClAP show state-of-the-art scores. OTOPE, even if better than current state-of-the-art, is not as accurate as ProClAP, showing weaknesses in embedding space use.

Our newly proposed approaches comparing microbiota samples based on the functional potential could pave the way for a better understanding of the links between microbiomes and their environment.

Gen abstract type

Poster

Section Type

AEM

68 - Environmental Matrix Comparison for Avian Influenza H5 Surveillance in Southern Saskatchewan

Raquel Mondragon[1], Jay Savaliya[1], Nicole Hansmeier[1], Tzu-Chiao Chao[1], Lilianne Gee[1]

[1]University of Regina

Avian influenza virus (AIV) surveillance traditionally relies on oropharyngeal and cloacal swabs from wild birds, although this approach faces logistical constraints in remote locations. Multiple environmental matrices, such as faeces, water, sediment and feathers have been proposed to close these surveillance gaps. However, the suitability of each sample type depends on wide range of local factors, from weather to bird migration patterns, and must be evaluated to ensure that the methods are fit for purpose.

This study compared viral RNA recovery across four environmental matrices, including surface water, sediment, bird feathers and feces collected from lakes in Southern Saskatchewan. Samples were collected between August and November 2025, coinciding with elevated detection of avian influenza by traditional surveillance. Water samples were concentrated via centrifugation or ultrafiltration and RNA was extracted using a range of RNA extraction kits for water, soil and fecal specimen. AIV presence was quantified using RT-qPCR and/or digital PCR targeting a H5 specific sequence within the hemagglutinin gene.

On average, feathers showed the highest average viral recovery (90.29 copies/mg) with a positivity rate of 29% (total N=49). Fecal samples yielded on average 2.3 cp/mg, with a positivity rate of 2% (total N=50). Positive surface water samples contained on average 1.6 cp/mL, with a positivity rate of 17% (total N=83). Only limited sediment samples were collected during this period, but positive samples exhibited an average viral concentration of 1.86 cp/mg.

Matrix performance varied by collection site and timing, but no correlation was observed between feather, and water sample viral load at a given site. Together, this study demonstrated that multi-modal sampling could assist in the detection of avian influenza in environmental reservoirs and could be an important tool to assist in avian influenza monitoring within a One Health framework.

Gen Abstract Type

Poster

Section Type

AEM

75 - Microbial Responses to Germanium Stress for the Identification of Selective Germanium-Binding Ligands

Aditya Kishor Barot[1, 2], Richa Verma[1, 2], Jessica Dougherty[1, 2], Mitchell Read Slobodian[1, 2], Sujeenthar Tharmalingam[1, 2], Vasu Appanna[1], Elina Taipova[1]

[1]School of Natural Sciences, Laurentian University, Sudbury, ON, P3E 2C6, Canada, [2]Medical Sciences Division, NOSM University, Sudbury, ON, P3E 2C6, Canada

Germanium is a strategically important critical metalloid widely used in fiber optics, infrared optics, semiconductors, and photovoltaic technologies. However, its dispersed occurrence in low-grade ores and industrial waste streams makes selective recovery challenging. Conventional extraction methods often lack specificity and require harsh chemical conditions. In contrast, biologically derived ligands offer a promising alternative for selective and sustainable metalloid recovery. Despite this potential, the interaction of germanium with microbial systems remains poorly understood, largely due to its neutral aqueous speciation as $Ge(OH)_4$, which limits uptake through conventional metal transport pathways.

This study investigates microbial responses to germanium exposure in a mixed microbial consortium grown under defined minimal media conditions. Cultures were subjected to varying germanium concentrations (0-5 mM) under both baseline (0.64 mM) and phosphate-limited (~35 µM) conditions to evaluate the role of nutrient stress in modulating metabolic responses. Microbial growth, metabolic activity, oxidative stress, and germanium distribution were systematically assessed using Bradford protein assays, pH monitoring, DNPH-based oxidative stress analysis, and inductively coupled plasma mass spectrometry (ICP-MS). Extracellular metabolite production was analyzed using reverse-phase high-performance liquid chromatography (HPLC).

Germanium exposure had minimal inhibitory effects on microbial growth, with biomass accumulation primarily influenced by phosphate availability rather than metalloid concentration. Cultures across all conditions reached stationary phase within ~42-72 h, and pH profiles indicated sustained metabolic activity, with only minor reductions observed at higher germanium concentrations. In contrast, phosphate limitation resulted in reduced biomass and increased oxidative stress, indicating that nutrient limitation is the primary driver of physiological stress in this system.

ICP-MS analysis revealed that 95-100% of germanium remained in the extracellular fraction, with negligible accumulation within microbial biomass. This suggests that germanium does not undergo significant cellular uptake and instead interacts predominantly within the extracellular environment. Under phosphate-limited conditions, extracellular protein production was not detected, indicating suppression of protein-based secretion pathways and a metabolic shift toward the production of low-molecular-weight extracellular metabolites.

HPLC analysis of spent culture fluid revealed the emergence of a distinct chromatographic peak at approximately 6.8 minutes that was absent in control cultures and increased significantly with increasing germanium concentration. This peak accounted for approximately 24% of the total chromatographic area at 1 mM germanium and ~60% at 5 mM germanium, demonstrating a strong dose-dependent response. The reproducibility and specificity of this peak suggest that it represents a germanium-responsive extracellular metabolite produced under metalloid stress.

Together, these findings indicate that microbial consortia respond to germanium exposure through extracellular metabolic adaptation rather than intracellular accumulation. The production of a distinct metabolite under phosphate-limited conditions supports the hypothesis that ligand-like compounds may be generated in response to metalloid stress, potentially influencing germanium speciation and reactivity. This work establishes a foundation for the identification and characterization of novel biologically derived ligands for selective germanium recovery, with ongoing LC-MS studies aimed at elucidating the molecular identity and functional role of the observed metabolite.

Gen abstract type

Poster

Section Type

AEM

86 - Investigating the Plausibility of Backward Contamination: A Pathogenicity Risk Assessment of Mars Analogue Sites

Kyle Story[1], Christina Davis[1], Zhixuan Feng[1], Jennifer Ronholm[2], Dao Nguyen[3], Maziar Divangahi[3], Lyle Whyte[1]

[1]Department of Natural Resource Sciences - McGill University, [2]Department of Animal Science - McGill University, [3]Department of Microbiology and Immunology - McGill University

Due to the range of conditions in which microorganisms survive on Earth, it has been speculated that microbial life could exist on other planetary bodies, such as Mars and the icy moons Europa and Enceladus. This search for life beyond Earth is an important focus of planetary exploration and a key feature in the designs of future missions. An example of this is the Mars Sample Return mission, which seeks to bring Martian sediments back to Earth, allowing the examination of Martian samples in greater detail than previously possible, including testing samples for signs of past or present life. While an exciting prospect, this mission carries the risk of backward contamination, where extraterrestrial life enters terrestrial ecosystems, acting as invasive species or pathogens. In the interest of planetary protection, and to provide some peace of mind for the public as to the risk this mission may pose, we have sampled remote Mars analogues, such as the saline cold springs on Axel Heiberg Island in the Canadian high Arctic and the McMurdo Dry Valleys of University Valley and Elephant Head in Antarctica, to assess the plausibility of risks to human health. Previously collected metagenome and genome sequencing data, along with metagenome-assembled genomes, were used for pathogenicity screens designed to search for genomic indications of potential pathogens. Specifically, pathogenicity islands were screened for using IslandViewer 4, and pathogenic gene products were screened for using the Center for Genomic Epidemiology's PathogenFinder and PathogenFinder2. Screens from Lost Hammer Spring, Gypsum Hill Spring, White Glacier, Atacama Desert, and Elephant Head have revealed potentially pathogenic organisms based on pathogenic protein family matches highlighted by PathogenFinder and PathogenFinder2, though the pathogenic gene products identified are not of great concern to public health. Analysis of Lost Hammer Spring, Gypsum Hill Spring, and Atacama Desert using IslandViewer 4 revealed no evidence of pathogenicity island structures within screened DNA sequences. Currently in progress are assessments of the phenotypic pathogenicity of both environmental analogue samples, and organisms isolated from these analogue sites. Through a series of growth experiments, over 150 organisms were successfully isolated from all analogue sites, with sixteen of these isolates observed to be capable of beta hemolysis. These sixteen hemolytic isolates were identified via Sanger sequencing of the 16S rRNA gene as various species of *Bacillus* or *Streptomyces*. These isolates, as well as various environmental samples from the analogue sites of interest to this study, will be carried forward into a lactate dehydrogenase cytotoxicity assay using HEK293 cells. This will allow for the further investigation of the phenotypic pathogenicity of samples and isolates taken from Mars analogue sites. Since these remote locations have little to no interaction with humans, we hypothesize that neither pathogenicity markers nor phenotypic pathogenicity will be observed to a degree that would pose a significant risk to public health, and this knowledge can in turn be applied to inform the handling of samples from other planetary bodies, such as those which will be brought back to Earth through the Mars Sample Return.

Gen abstract type

Poster

Section Type

AEM

96 - Biodegradation of Phenolic Pollutants in Saline Wastewater Using Tyrosinase-Producing Halophilic Bacteria

Nawab Ali[1]

[1]Abdullah Al Salem university

Phenolic compounds are among the most hazardous environmental pollutants commonly found in industrial wastewater, particularly in wastewater from the petrochemical, pharmaceutical, and dye manufacturing industries. Due to their high toxicity, persistence, and resistance to conventional treatment processes, effective removal strategies are required to mitigate their environmental impact. Microbial biodegradation has emerged as an eco-friendly, cost-effective alternative for removing phenolic contaminants, especially under extreme environmental conditions. This study aimed to isolate and characterize tyrosinase-producing halophilic bacteria capable of degrading phenol in saline wastewater environments. Bacterial isolates were obtained from saline soil and water samples and screened for tyrosinase production using enzyme activity assays. Isolates showing high enzymatic activity were further characterized through morphological, biochemical, and molecular approaches, including 16S rRNA gene sequencing for taxonomic identification. The isolated bacterial strains were identified as *Bacillus flexus* and *Bacillus subtilis*. The phenol-degrading potential of the selected strains was evaluated under varying environmental conditions, including salinity, pH, and temperature. The results revealed that several halophilic bacterial isolates exhibited significant tyrosinase activity and efficiently degraded phenol even under high salinity conditions. These findings highlight the potential of tyrosinase-producing halophilic bacteria as promising biocatalysts for treating phenol-contaminated saline wastewater. The study provides insights into sustainable microbial approaches for bioremediation of industrial effluents and supports the development of environmentally friendly wastewater treatment strategies.

Gen abstract type

Poster

Section Type

AEM

98 - Exploring A Subsect of the Microbial Diversity of Manitoban Saline Springs: Isolation of Novel Roseobacteraceae Aerobic Anoxygenic Phototrophs

Caleb Pereira[1], Katia Messner[1], John Kyndt[2], Marike Palmer[1], Vladimir Yurkov[1]

[1]University of Manitoba, [2]Bellevue University

Anoxygenic photosynthesis, a mode of bacterial light harvesting, was thought to be an exclusively anaerobic process for a long period of time. This was until the discovery of strains capable of doing so in the presence of oxygen, named aerobic anoxygenic phototrophs (AAP). Fundamentally, they differ from other anoxygenic phototrophs in being obligate aerobes, are not considered autotrophs, and cannot fix CO2 due to the lack of RuBisCo. Photosynthesis in AAP is often utilized in nutrient-limited environments, albeit as a supplemental energy source, to outcompete strict heterotrophs. Intriguingly, synthesis of the photo complexes occurs in the dark to combat photo-oxidative stress enhanced by radicals formed during production of bacteriochlorophyll, a pigment essential to converting light energy into ATP.

Hypersaline environments pose a great deal of intrigue for biodiversity research, as life of any kind is restricted to those adapted to such conditions. As a result, these habitats are often confined to microorganisms, leading to studies focused on stagnant-water habitats: marshes, lakes, and ponds. Yet comparatively, little work has been done on flowing waters.

A particular area of interest is the concentration of low-temperature salt spring systems near Lake Winnipegosis in West-Central Manitoba. Given the region's anthropogenic isolation and its connection to astrobiology, serving as an analog for the study of microbial life on Mars, it presents as an exciting candidate for microbial studies.

The alphaproteobacterial family *Roseobacteraceae* comprises a diverse array of photo- and chemoheterotrophs originating from marine, hypersaline, and terrestrial environments. Large proportions of them have been isolated from high-salt areas, with many requiring it for growth. Two strains, MS613 and MS785, were isolated from the spring system and identified as AAP due to being obligate aerobes, incapable of autotrophic growth, and the production of bacteriochlorophyll a incorporated into the photosynthetic reaction center and light-harvesting complex. Physiological tests confirmed that both are alkalitolerant and halotolerant, preferring saline conditions, and withstanding concentrations up to 10-12% NaCl. Notably, MS785 appears psychrotolerant, as growth could occur at temperatures as low as 4°C. Based on 16S rRNA sequences, MS613 and MS785 were categorized as strains in the genera *Meridianimarinicoccus* and *Roseovarius*, respectively. MS613 was closest related to *M. zhengii* (sequence similarity of 98.53%), *M. roseus* (97.87%), and *Tropicimonas omnivorans* (96.03%). MS785 was most closely related to *R. autotrophicus* (98.25%), *R. mucosus* (96.62%), and *R. nitratireducens* (96.42%). Genomic comparisons, using average nucleotide identity and digital DNA-DNA hybridization, against the closest related for both fell below the established cutoffs for species designation (<95% and <70%, respectively). The phylogenomic tree of isolates and closely related species in *Roseobacteraceae* shows each strain forms a distinct branch within its genus, further supporting the classification as a new species.

Due to their roles in local carbon and energy cycling, AAP are an important member in spring communities. Isolation and characterization of these two novel strains provide greater insight into the microbial diversity of such environments, particularly regarding the presence of anoxygenic phototrophs, as well as expand known habitats of *Roseobacteraceae*.

Gen abstract type Poster

Section Type

AEM

99 - *Apolloniradiicaulis salifontis* gen. nov., sp. nov., a Novel Prosthecae Aerobic Anoxygenic Phototroph Which has Extensive Photosynthetic Complex Production

Katia Messner[1], Caleb Pereira[1], John Kyndt[2], Marike Palmer[1], Vladimir Yurkov[1]

[1]University of Manitoba, [2]Bellevue University

Caulobacterales have long been known as a distinct group of Alphaproteobacteria which display a dimorphic lifestyle consisting of a motile flagellated and a reproductive non-motile phase. A key trait of the later stage is the production of prosthecae, an extension of the cell envelope, which increases the cells surface area supporting increased nutrient uptake and serving as the site of cell attachment to surfaces, commonly through the production of an adhesive. In some members of the order, such as *Maricaulaceae* and *Caulobacteraceae*, there are species which are identified as aerobic anoxygenic phototrophs (AAP). This group of light-harnessing microorganisms uses a cyclic pathway to produce chemical energy in the form of ATP that does not produce oxygen as byproduct. The most important components are the light harvesting complex(es), which absorb light and the reaction center, where photon energy is directed to for pathway initiation. Unlike other anoxygenic phototrophs, AAP are unable to grow autotrophically or anaerobically. Nonetheless, it provides them a competitive advantage over strict heterotrophs, especially under nutrient-limited conditions. Since photosynthesis is used as an additional energy source to aerobic respiration, AAP typically synthesize less photosynthetic complexes relative to other phototrophs that are more dependent on this process. Prosthecate strain MS644^T isolated from saline springs is distinct both in physiological features and phylogenomic placement within *Maricaulaceae*. It is a moderate, obligate halophile that can grow up to 16% NaCl, making MS644^T the most halophilic member of the family and can grow in neutral to alkaline conditions (pH 10). The strain can neither grow in the absence of oxygen nor can it live autotrophically. However, MS644^T expresses an anoxygenic photosynthetic apparatus and is therefore an AAP. Notably, an extensive amount of light-harvesting I complex, reaction center and photosynthetic pigment bacteriochlorophyll *a* relative to carotenoids, are expressed. Usually in AAP it is the opposite, where carotenoids are produced in surplus compared to bacteriochlorophyll *a* to combat photooxidative damage. This suggests photosynthesis in MS644^T comprises a greater portion in total energy production. Species delineation tools like digital DNA-DNA hybridization and average nucleotide identity were below standard species cutoffs (< 70% and 95% respectively). Furthermore, average amino acid identity values of MS644^T and closely related species fell within the genus delineation range in *Maricaulaceae*. Therefore, strain MS644^T was recently classified as a new genus and species with the name *Apolloniradiicaulis salifontis*. The discovery of a new phototroph expands known AAP and diversity in *Caulobacterales*. The unusual, enhanced light-harvesting I and reaction center complex production supports further exploration into the role of photosynthesis in strain MS644^T's and other prosthecate AAP's biphasic lifecycle.

Gen abstract type

Poster

Section Type

AEM

101 - Fast and quantitative detection of *Salmonella* and *E. coli* O157 in milk using electrochemical biosensing

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While numerous biosensors have been reported for foodborne pathogen detection, most remain focused on binary outputs or limit-of-detection (LOD) metrics and fail to provide robust, interpretable quantification directly in complex dairy matrices. Here, we report a matrix-adaptive electrochemical biosensing framework that enables both rapid screening and decision-oriented enumeration of *Escherichia coli* O157 (*E. coli* O157) and *Salmonella* spp. directly in milk. The platform employs reduced graphene oxide (rGO)-based immunosensors operated under low-voltage excitation and integrates a voltage-threshold-based interrogation strategy to translate electrochemical responses into screening-relevant concentration ranges rather than relying on potentially misleading absolute CFU reporting. The biosensors achieved ultra-low limits of detection of approximately 0.02 CFU mL^{-1} for *E. coli* O157 and 0.003-0.005 CFU mL^{-1} for *Salmonella* spp., well below regulatory absence thresholds. By coupling sensitive electrochemical detection with a robust enumeration framework tailored to complex matrices, this work moves beyond LOD-centric biosensing and provides a practical approach for rapid, on-site food safety screening of milk. The proposed strategy supports early intervention and informed decision-making and can be extended to other challenging food and environmental samples.

Gen abstract type

Poster

Section Type

AEM

108 - Influence of Acid Pre-Exposure on Cell Wall Remodelling and Resistance Evolution in *Listeria monocytogenes*

Serena Lam[1], Rhea Azavedo[1], Veronica Guariglia[1]

[1]Western University

Listeria monocytogenes (*Lm*) is a Gram-positive foodborne pathogen responsible for listeriosis, a serious infection with high hospitalization rates and a mortality rate of 20-30%. *Lm* presents a major challenge to both public health and the food industry because of its ability to tolerate environmental stresses used in food preservation, including refrigeration, high salt, and low pH. These adaptations allow *Lm* to persist in ready-to-eat foods and food-processing environments, leading to costly recalls and outbreaks. During food production, acidification is frequently used to achieve the desired properties of products such as soft cheeses; however, exposure to acidic environments can also trigger bacterial stress responses that enhance survival. Previous work from our group has demonstrated that the cell envelope modifications involving the D-alanylation of lipoteichoic acids (by DltABCD) and L-lysinylation of the bacterial membrane (by MprF) contribute to acid-induced, antimicrobial resistance in *Lm*, evidenced by a higher resistance to antimicrobials in cells grown at acidic pH.

Here, we sought to define how acidic environments reshape the *Lm* cell envelope and drive antimicrobial resistance. We hypothesized that acid exposure induces modifications to the *Lm* cell envelope that increase resistance to cell wall-targeting antimicrobial strategies. To address this, a transcriptomic analysis was performed using cells grown in neutral or acidic conditions prior to exposure to nisin. RNA-seq data indicate that nisin treatment under acidic conditions upregulate the *dltABCD* operon, further suggesting that cell envelope modifications play an important role in the acid stress response. Furthermore, mutant strains targeting key cell envelope modification genes (*dltA* and *mprF*) and its regulator (*virR*) were used to evaluate susceptibility to antimicrobial compounds, while parallel evolution experiments were conducted to track the emergence of resistance under sustained acidic and antimicrobial stress. We observe increased resistance to nisin under acidic conditions, while mutants lacking key cell envelope modification genes exhibit increased susceptibility. Furthermore, acid pre-exposure enhances nisin resistance in wild-type strains, and prolonged exposure to sublethal nisin concentrations leads to increased resistance during directed evolution experiments. Together, these findings suggest that acid exposure primes *Lm* for enhanced resistance to cell wall-acting antimicrobials. These data support our hypothesis and demonstrate that acidic conditions directly influence antimicrobial susceptibility.

Collectively, this work aims to elucidate the molecular mechanisms by which acid stress alters the *Lm* cell envelope and enhances resistance to food-grade antimicrobial peptides. These insights will improve our understanding of how food-processing conditions shape pathogen adaptation and will inform the development of more effective antimicrobial strategies to limit *Lm* persistence and reduce the risk of foodborne disease.

Gen abstract type Poster

Section Type AEM

109 - Strain-specific optimization of bacteriocin production by replacing conventional media components with dairy and maize co-products

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Ionophore antibiotics, such as monensin, are widely used in North American ruminant production to modulate rumen fermentation and improve feed efficiency, but growing concerns with the dissemination of antibiotic resistance in the livestock environment have created demand for natural alternatives. Bacteriocins, ribosomally synthesized antimicrobial peptides produced by lactic acid bacteria (LAB), offer promise as next-generation feed additives that may selectively influence rumen microbial balance while enhancing feed safety. The overall hypothesis is that nisin and pediocin-like peptides could inhibit undesirable Gram-positive species, including *Listeria monocytogenes*, without broadly suppressing beneficial populations in the rumen, potentially supporting improved fermentation dynamics and reduced methane emissions. However, large-scale bacteriocin production is limited by the cost of standard laboratory media, motivating investigation into sustainable, by-product-based culture systems. This study aimed to identify strain-specific, cost-effective media and culture conditions that maximize bacteriocin production by *Lactococcus lactis* UL719 and *Pediococcus pentosaceus* LL699 for use in rumen-directed feed formulations. Following screening of 31 LAB isolates, these two strains were selected for superior antimicrobial activity. Each was evaluated across 18 media types, including M17, MRS, Elliker, and whey permeate (WP) formulations supplemented with corn steep liquor (CSL, 0-10%), yeast extract (YE, 0-5%), and glucose (2%) at 30 °C. Antimicrobial activity, expressed as arbitrary units per mL (AU/mL), was assessed using microtiter inhibition assays against *Pediococcus acidilactici* 1CP28 (indicator for nisin activity) and *L. monocytogenes* ATCC 15313 (indicator for pediocin activity). Time-course batch fermentations with selected formulations (M17, Elliker, WP + 5% CSL for *Lc. lactis*; and WP + 2% CSL for *P. pentosaceus*) were monitored for growth, pH, and activity over 60 h. *Lc. lactis* UL719 exhibited growth-associated, nisin-like activity detectable from 10-12 h, peaking at 24 h and decreasing thereafter. M17 supported the highest titres (1024 AU/mL; 9.3-9.7 $\log_{10}$ CFU/mL). Although WP alone yielded negligible activity, supplementation with 5% CSL improved titres to 256-512 AU/mL while sustaining high viable biomass, representing a cost-efficient option when live-cell delivery is desired. YE supplementation enhanced titres in WP systems, whereas glucose addition reduced activity, likely due to acid accumulation and possibly catabolite repression. *P. pentosaceus* LL699 exhibited stationary-phase-linked pediocin-like production, with weak activity at 24 h that increased sharply by 48 h. WP enriched with low CSL (1-2%) or YE (2-5%) supported the highest titres (2048 AU/mL in screening; 4096 AU/mL in optimized WP + 2% CSL), exceeding those in Elliker (1024 AU/mL) and M17 (512 AU/mL). In WP + 2% CSL, LL699 reached 8.8 $\log_{10}$ CFU/mL and sustained ≥ 512 AU/mL from 18-60 h without requiring peptide desorption from cell surfaces. Peak activity for both strains corresponded to pH 4.2-4.9, consistent with improved peptide stability under mildly acidic conditions. Altogether, WP-CSL media can be tailored to individual LAB strains for efficient bacteriocin production. Nisin production by *Lc. lactis* UL719 was highest in M17, while *P. pentosaceus* LL699 achieved superior, persistent pediocin-like synthesis in low-CSL whey permeate. These findings support the feasibility of utilizing dairy and maize by-products as scalable, economical substrates for producing bacteriocins and microbial biomass intended for sustainable dairy cattle nutrition. Perspectives include developing pH-controlled fermentation, which could further increase bacteriocin yield.

Gen abstract type Poster **Section Type** AEM

115 - Modeling vaginal microbiota interactions to understand antibiotic resistance

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The vaginal microbiota represents a dynamic and heterogeneous ecosystem in which commensal bacteria and opportunistic pathogens coexist and interact. These interspecies interactions profoundly influence microbial behavior, including virulence, metabolic activity, and susceptibility to antimicrobial treatments. Understanding how these relationships shape community-level responses is essential for developing more effective therapeutic strategies.

To better capture microbial behavior under clinically relevant conditions, we model the vaginal environment *in vitro* to study bacterial responses within a physiologically meaningful context. Traditional approaches to pathogenesis and antibiotic resistance have largely relied on isolated bacterial cultures under laboratory conditions that fail to reflect the complexity of natural microbial ecosystems. *In vivo*, microorganisms predominantly exist within structured, multispecies communities known as biofilms, where environmental cues and interspecies interactions strongly influence their behavior. Biofilms provide access to nutrients and protection against environmental stressors, including antimicrobial agents. However, interactions within polymicrobial biofilms remain insufficiently understood, particularly regarding their contribution to antibiotic resistance, highlighting the need for more representative experimental models.

In this project, we aim to determine how vaginal microorganisms cooperate and interact to enhance antibiotic resistance within a community context. To achieve this, we are developing an in vitro vaginal biofilm model that recapitulates key features of the natural environment, enabling the study of microbial organization, structure, and function under controlled conditions. Using this model, we assess both antibiotic resistance and tolerance at the community level. Our preliminary observations reveal that different vaginal species form biofilms with distinct structural organizations, suggesting that biofilm architecture is species-dependent and may influence antimicrobial susceptibility. Furthermore, in dual-species biofilms, the opportunistic pathogen *Staphylococcus aureus* tends to dominate the phenotypic characteristics of the biofilm, indicating a strong capacity to shape community structure and function. This dominance may have important implications for the overall resistance profile of the microbial community.

By integrating polymicrobial interactions into the study of antimicrobial resistance within a modeled vaginal environment, this research will contribute to a deeper understanding of how microbial community structure and interspecies interactions influence antibiotic efficacy, offering new perspectives for the development of targeted and effective therapeutic approaches.

Gen abstract type

Poster

Section Type

AEM

116 - Assembly-Free Metagenomics Profiling Coupled with Metabolic Network Analysis for Soil Remediation

Harbinder Kaur[1], Namrata Joshia[1], Andrew Lynn[1]

[1]Jawaharlal Nehru University

Natural disasters and industrial activities such as mining, manufacturing, and intensive agriculture can cause severe and often irreversible soil degradation. Healthy soils represent complex, dynamic ecosystems in which geological, hydrological, and biological components are tightly coupled through metabolic interactions. Effective remediation, therefore, requires a systems-level understanding of how microbial community function is disrupted and how it may be restored.

We have developed a network-based framework for assembly-free metagenomic analysis that rapidly identifies functional and taxonomic profiles in soil metagenomic and metatranscriptomic data. The framework employs a custom reference database built from curated Ensembl transcript sequences. By linking custom database entries to a locally built functional lookup table, we enable direct linkage between taxonomic identity and gene-level function. By generating a weighted functional abundance matrix directly from read-level data, the pipeline bypasses the complexities of transcript reconstruction, providing a faster approach that overcomes challenges such as the fragmented nature of complex microbiome datasets. This approach was motivated by our earlier work on post-coal mining soils, in which assembly-based methods consistently recovered very low functional activity.

These functional profiles are mapped onto a global metabolic reference network, enabling quantitative assessment using topological measures such as network connectivity, modular integrity, and pathway erosion. We further apply a network expansion algorithm to predict targeted combinations of microbial taxa that can act as functional “bridges,” restoring metabolic reachability. We benchmark this framework against a published Auto-Brewery Syndrome (ABS) metagenomic dataset that exhibits clear shifts in microbiome organization and ethanol-associated metabolic activity, making it ideal for evaluating sensitivity, functional recovery, and biological consistency. The approach is validated using simulated metagenomic datasets representing intact and degraded soil microbial communities, demonstrating its potential as a predictive tool for guiding microbiome-based soil remediation strategies.

Gen abstract type

Poster

Section Type

AEM

120 - Non-canonical nitrogen metabolism by comammox *Nitrospira* and ammonia-oxidizing archaea within rotating biological contactors of a municipal wastewater treatment plant

Michelle`` McKnight[1], Alexander Umbach[1], Bram Vekeman[2], Shankar Kundapura[2], Georg Mlynek[2], Hanna Koch[2], Kristina Djinovic Carugo[3], Laura Lehtovirta-Morley[4], Michael Wagner[2], Josh Neufeld[1]

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Rotating biological contactors (RBCs) at a municipal wastewater treatment plant host nitrifying biofilms dominated by complete ammonia oxidizing (comammox) *Nitrospira* and the ammonia-oxidizing archaea (AOA) representative *Candidatus* Nitrosocosmicus hydrocola. Metatranscriptomic analyses of RBC biofilms revealed high expression of ammonia oxidation genes for both nitrifier groups, confirming active nitrification under ammonia-limited conditions. In addition, comammox *Nitrospira* expressed diverse ureohydrolase superfamily genes, including ureases, cyanate metabolism genes, and putative agmatinases/guanidinases/arginases, indicating capacity to generate ammonia from alternative nitrogenous compounds. We observed that *Ca.* N. hydrocola exhibited exceptionally high transcription of a putative “proclavaminate amidinohydrolase” with homology to the recently described guanidinase from comammox *Nitrospira*, at levels comparable to ammonia monooxygenase genes across biofilms, although these transcripts were not detectable in a corresponding ammonia-fed enrichment culture. Cultures of *Ca.* N. hydrocola and *Nitrososphaera viennensis* (encoding a homologue of the “proclavaminate amidinohydrolase”), were grown with ammonia or guanidine as the sole energy source. Guanidine-amended incubations depleted guanidine and produced nitrite, with elevated transcript abundance relative to ammonia-grown cultures, confirming that guanidine can support archaeal nitrification. Predictions using AlphaFold demonstrated conservation of the tertiary structure and most key amino acid residues within the active site relative to the guanidinase of comammox *Nitrospira*. Further research will test AOA growth on guanidine and the substrate range of this enzyme. Our results identify guanidine metabolism as a previously unrecognized pathway for AOA and reinforce how both comammox *Nitrospira* and some AOA can exploit alternative nitrogen compounds to sustain nitrification.

Gen abstract type

Poster

Section Type

AEM

121 - Inhibitors of GlpG rhomboid protease reduce antimicrobial resistance of uro-pathogenic bacteria found in kidney stents biofilm

Davier Gutierrez-Gongora[1], Jimmy Lu[1], Ella Corry[1], Elena Arutyunova[1], Joanne Lemieux[1]

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Bacterial infections kill approximately five million people each year worldwide. One in five of these deaths is directly attributable to antimicrobial-resistant (AMR) pathogens, a higher toll than HIV or malaria. In Canada, one in four human bacterial infections resist current antibiotics. Targeting virulence factors, which disable pathogens with less selective pressure for resistance, presents a promising alternative. For example, pili, hair-like extracellular structures, are crucial for adhesion and host invasion. Our lab previously identified a serine transmembrane rhomboid protease, GlpG, which is essential for FimA maturation (the main component of type I pili), bladder infection, and biofilm formation in uro-pathogenic *Escherichia coli* (UPEC). Although GlpG is conserved in prokaryotes, its function in other pathogenic bacteria, such as *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa,* which also demonstrate significant AMR*;* remains unclear. Some of these pathogens form biofilms on bladder stents, enabling nutrient acquisition and progressive AMR which threatens the patient's development. Here, we isolated and identified several bacterial species from clinical kidney biofilm stents using microbiological and molecular techniques. We then characterized their capacity to form single- and multispecies abiotic biofilms and determined their resistance profiles to common antibiotics. We evaluated whether *E. coli*-GlpG inhibitors could block analogous rhomboid proteases in *S. aureus*, *P. aeruginosa,* and *K. pneumoniae,* and assessed the impact of these inhibitors on biofilm formation and their potential synergy with approved antibiotics. Notably, GlpG inhibitors suppress biofilm formation without killing bacteria, underscoring their antivirulence effect. We also used FLAG-tagged UPEC-GlpG for affinity pulldown and mass spectrometry to identify GlpG's substrates and interactors before and after host invasion. Future work will involve characterizing additional rhomboid proteases in pathogens beyond *E. coli*. This research demonstrates the promise of GlpG-like protease inhibitors as new broad-spectrum antimicrobials that target virulence and are less likely to drive AMR.

Gen abstract type

Poster

Section Type

AEM

122 - The roles of tripartite efflux pumps in antibiotic resistance and phage infection cycle in *Acinetobacter baumannii*

Chloé Girouard[1], Olivier Perreault[1], Alexandre Gagné[1], Alicia Benoit[1], Louis-Charles Fortier[1], Evan Mercier[1]

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Acinetobacter baumannii is a gram-negative pathogen associated with deadly nosocomial infections, such as ventilator-associated-pneumonia. This pathogen possesses several virulence factors and antibiotic resistance mechanisms, including tripartite efflux pumps, membrane transport proteins involved in the efflux of antibiotics and components involved in virulence. Tripartite efflux pumps are known to confer multi-drug resistance (MDR) in *A. baumannii* isolates, which poses a great risk to the general population. To counter this rise in antibiotic resistance, efforts are being poured in research centered towards bacteriophages therapy to treat MDR *A. baumannii* infections. In *A. baumannii*, phage therapy can be rendered ineffective by the elimination of the phage's main receptor, but research has shown that this elimination results in a loss of antibiotic resistance and certain virulence factors. To date, the capsule appears to be the main receptor for *A. baumannii* phages, but in other species, like *Escherichia Coli,* phages have shown to use efflux pumps as receptors. Our hypothesis is that tripartite efflux pumps play an important role in antibiotic resistance and virulence in *A. baumannii* and potentially in the phage infection cycle.

Our first objective centers around the creation of tripartite efflux pumps knock-outs (KO) and the study of the subsequent phenotypes and genetic changes that derive from the elimination of pump subunits. Knock-out (KO) models of the outer membrane channel and inner membrane transporter were engineered for 6 pumps (AdeIJK, AdeABC, AdeFGH, MacAB-TolC, ModABC and YbhFRS) in the ATCC 17978 strain through CRISPR-Cas9 technology. Minimal inhibitory concentration assays have shown a decrease in antibiotic susceptibility for several different classes of antibiotics, with the strongest decrease found in the KO models *ΔadeK* and *ΔadeJ.* Motility assays showed a severe decrease in motility in several of our KO models compared to the WT strain, as well as quantitative changes in biofilm formation. We also performed 5-days and 10-days laboratory evolution to Tigecycline, with half of our KO strains, and preliminary results show that our KO models have a harder time adapting to the increasing concentrations of Tigecycline compared to the WT model, but all models reached Tigecycline resistance over the span of 10 days. We plan to reproduce these experiments with the other models, as well as study the phenotypes associated with adaptive evolution. DNA and RNA were extracted from cultures to study changes in gene composition and expression through sequencing.

The second objective involves testing the roles of efflux pumps in phage infection. Early screening in ATCC 17978, identified 8 phages that infected this strain. We plan to test if our selected efflux pumps are involved in the infection cycle of these phages, as well as screen for the potential of phage-antibiotic synergy.

These results help paint a picture of the importance of efflux pumps in *A. baumannii*'s survival, virulence, and resistance to antibiotics. This project will help our understanding of the roles of tripartite efflux pumps in *A. baumannii,* and to determine if the outer membrane channels of efflux pumps could be good targets for phage therapy.

Gen abstract type Poster

Section Type AEM

123 - Investigation of Bcc Phage Carl1's Type VI Secretion System Related Moron Genes

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The *Burkholderia cepacia* complex (Bcc) is a group of opportunistic, pathogenic bacteria with high levels of antibiotic resistance that can cause serious, if not lethal, infections in people with cystic fibrosis and chronic granulomatous disease. These traits make the Bcc a candidate for bacteriophage (phage) therapy. Carl1 is a myovirus, P2-like phage that was isolated from a soil sample using clinical isolate *Burkholderia cenocepacia* strain Van1, a member of the Bcc, and is currently being investigated as a potential therapeutic agent. Through genomic analysis of Carl1, a moron gene cluster was found containing three genes related to bacterial type VI secretion systems (T6SS). Moron genes, or genes carried by phages that do not play a specific role in phage function, have been known to change the physiology of their bacterial host. Therefore, it is essential to elucidate their effects on the host bacterium before use of the phage as a therapeutic agent. Based on BLASTp alignments and Geneious InterPro Scan conserved domain search, these genes encode proteins homologous to a type VI secretion tip protein (gp47), a VRR-Nuc domain containing protein (gp48), and a type VI immunity family protein (gp49). Despite also containing lysogeny related genes, such as a gene encoding a tyrosine-type integrase (gp46), no stable lysogens of Carl1 have yet been isolated. Thus, to test the function of these moron genes, they were cloned onto a plasmid in the phage Carl1 host *B. cenocepacia* strain K56-2. Preliminary evidence suggests that at least one of these genes is toxic to the host cell, as no transformants with the entire gene cluster have been successfully isolated. The VRR-Nuc domain (IPR014883) has been shown to possess nuclease activity that can function as a T6SS effector, identifying gp48 as the likely candidate for the lethal gene product, however, studies have shown that the type VI secretion tip proteins can also carry effector domains in their C-terminals. In contrast, gp49 contains a TsiV domain (IPR021815) known to bind VRR-Nuc domain containing effectors, neutralizing them, suggesting that gp49 would likely not be lethal. Currently, work is being conducted to clone the moron genes individually, as well as the whole cluster and gene combinations under an inducible promoter, to identify which gene product is lethal. It is suspected that the putative lethality of this gene cluster could explain the lack of lysogens, and in addition to natural phage killing of the host bacterium, could provide a secondary killing mechanism further expanding phage Carl1's potential as a therapeutic agent.

Gen abstract type Poster **Section Type** AEM

126 - Diversity of holdfast-mediated adhesion in environmental Alphaproteobacteria from Québec aquatic environments

Cecile Berne[1], Lorine Lardinois[1], Marin Debidour[1], Matteo Paniconi[1], Nathaniel Danis[1], Emily D. Sprowls[2], Kathryn Kavanagh[3], Laura Gilbert[3], Yves V Brun[1]

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Biofilms are complex microbial communities that enable bacteria to attach to surfaces and thrive in diverse environments. While they fulfill critical ecological functions, they also contribute to biofouling, contamination, and antimicrobial resistance, posing significant economic and health challenges. In Alphaproteobacteria, irreversible surface colonization is often initiated by polar adhesins such as the *Caulobacter* holdfast, a nanoscale adhesive notable for its exceptional strength, particularly under water.

Here we characterized the natural diversity of holdfast-mediated adhesion across a collection of environmental strains isolated from Québec freshwater samples collected through a citizen science initiative conducted in partnership with Water Rangers. Isolates were screened for biofilm formation and polar attachment, and a representative subset was further analyzed using phenotypic assays, fluorescence microscopy, and whole-genome sequencing. Phylogenetic analysis revealed that most isolates belong to the Caulobacterales, a group known to use polar adhesion and holdfast production as key strategies for early surface colonization and biofouling. Integration of comparative genomics with phenotypic and microscopy data uncovered substantial intra-order variation in holdfast composition and adhesive properties.

We further evaluated biofilm formation on a panel of newly developed polymer brush coatings designed as sustainable antifouling materials for maritime applications. Three formulations consistently inhibited bacterial attachment across both laboratory strains and environmental isolates, without affecting cell viability. These findings represent an initial step toward the development of non-toxic antifouling strategies effective against bacterial adhesion across ecologically relevant species.

Together, this work provides a framework for the rational design of bio-inspired underwater adhesives and the development of next-generation antifouling materials with demonstrated efficacy across diverse environmental bacteria.

Gen abstract type

Poster

Section Type

AEM

129 - Exploring Lipid Production in an Environmental *Hyphomicrobium* isolate: Sustainable Lipid Platforms for Hydrocarbon Production

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[1]University of Alberta

Global efforts have focused on the development of sustainable sources of fuels, chemicals, and materials. Lipids are a versatile feedstock, offering a renewable source of carbon that can be converted into commodities like biofuels. Microorganisms offer a promising route to produce products like lipids due to their low environmental footprint, genetic engineering potential, and ability to grow on diverse feedstocks. Methylotrophs, which can utilize methanol as a sole carbon source, are particularly promising to produce lipid-derivatives because methanol is abundant, cost-effective, and can be produced renewably.

An environmental isolate of a *Hyphomicrobium* species (OSCF32) has shown to accumulate lipids and consume methanol. Conventional fatty acids like oleic acid have been investigated in lipid pyrolysis technology to produce fatty acid-derived biofuels. Fatty acids provide good biofuel precursors due to their carbon: hydrogen ratios and structural similarity to petroleum hydrocarbons. However, carbon storage through polyhydroxyalkanoate (PHA) accumulation competes with fatty acid synthesis, as these pathways share metabolic intermediates like acetyl-CoA and reducing agents. This research aims to address these challenges through three objectives: modifying growth parameters to influence lipid profiles of OSCF32, engineering OSCF32 for increased lipid production and the conversion of microbial lipids into biofuels using non-catalytic pyrolysis. Changing growth variables like pH can provide insight on how a multi-faceted approach (both genetic modifications and growth conditions) can be used to achieve high lipid production.

Preliminary results show that sublethal acid stress diverts carbon toward fatty acid synthesis and increases lipid accumulation. Sublethal pH followed a hormetic pattern, where low pH adjustment led to significantly higher lipid accumulation with no negative consequences on biomass. A pH of 6.4-6.5 exhibited the highest lipid accumulation, nearly double the lipid content when compared to pH 6.8 (control). When pH < 6.4, growth of OSCF32 was hindered, and lipid accumulation also decreased. At a pH < 6.3, no PHA accumulation was observed, generating a feedstock composed exclusively of fatty acids. By exploring the effect of extrinsic growth conditions on lipid production in OSCF32, this novel species can be characterized to expand the types of chassis used for bioproduction.

By creating an engineered OSCF32 strain and a scalable pipeline, this project aims to establish a sustainable, microorganism-based route to hydrocarbon fuels. The outcomes will advance Canada's position in renewable energy innovation, reduce greenhouse gas emissions, and contribute tangible solutions for a cleaner future in Canada and the rest of the planet.

Gen abstract type

Poster

Section Type

AEM

130 - Isolation and characterization of *Burkholderia cepacia* complex-targeting phages from a wastewater sample

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Individuals with Cystic Fibrosis (CF) are vulnerable to devastating lung infections caused by Gram-negative, opportunistic pathogens of the *Burkholderia cepacia* complex (Bcc). In some individuals, these infections escalate to "*cepacia* syndrome," which is a severe, life-threatening condition. The standard treatment for Bcc infection is triple-combination antibiotic therapy; however, these treatments are inadequate at eradicating the bacteria, and thus chronic or recurrent infection is highly prevalent among individuals with CF. An alternative treatment for Bcc infection is desperately needed to combat issues of bacterial resistance and improve the quality of life and lifespan of individuals with CF.

In CF patients with antibiotic-resistant Bcc infections, early clinical reports have documented improved lung function and reduced bacterial burden following phage therapy. Phages (bacteriophages) are viruses that infect bacterial cells, replicate within them, and destroy the host cell to release their progeny. Phages are naturally found in the environment and are the most abundant biological entity on Earth. Phage therapy uses these phages to treat bacterial infections. Phage cocktails are a type of phage therapy that combine multiple phages with different infection mechanisms to improve treatment effectiveness and reduce the likelihood of single mutations allowing the bacterium to acquire resistance. Although some phages active against strains of the Bcc have been characterized previously, more work is needed to discover and characterize additional Bcc-targeting phages to expand our phage bank, enhance phage cocktails, and reduce the likelihood of treatment resistance.

Phages require a host bacterium for replication, so they should be found where the bacterial host is located. Building upon previous studies identifying Bcc strains in wastewater samples, the research described aims to discover and characterize novel Bcc-targeting phages from a wastewater sample collected in Edmonton, Alberta. Using several clinically isolated strains of Bcc as "bait" and two unique wastewater protocols, seven potentially novel phages have been discovered targeting six different strains of Bcc. Purification, amplification, and genome extraction have enabled the identification of these phages by DNA sequencing, and host-range analysis has provided additional information on their utility for use in phage therapy/cocktails. The implication of this research is that novel Bcc phages may be isolated from wastewater, and incorporated in phage cocktails to improve treatments for Bcc infections in individuals with CF.

Gen abstract type

Poster

Section Type

AEM

132 - Characterizing a new class of Aminoglycoside-Inactivating Kinases

Amna Abbas[1], Kalinka Koteva[1], Gerard Wright[1]

[1]McMaster University

Antimicrobial resistance (AMR) continues to rise globally on an alarming scale, raising the need for proactive identification of resistance determinants before their emergence in clinical pathogens. Functional metagenomics has enabled the discovery of novel antibiotic resistance genes (ARGs) from environmental reservoirs; however, conventional approaches are biased toward abundant, previously characterized genes and often fail to prioritize mobile elements with high dissemination potential. Integron-targeted functional metagenomics addresses these limitations by enriching for gene cassettes associated with horizontal gene transfer. Using this approach, Bohm et al. recently identified the gene *gar* as a putative aminoglycoside resistance determinant, though its mechanism of action remained unknown (1). A particularly striking finding of this study is that gar was already present in multiple clinical isolates prior to its discovery, including multidrug-resistant strains of *Pseudomonas aeruginosa, Luteimonas sp.,* and *Salmonella enterica* from geographically distinct regions (1). The presence of *gar* across diverse pathogens and continents further indicates that it has already undergone horizontal transfer and may continue to disseminate

In my work, we functionally and biochemically characterize *gar*, establishing it as a gentamicin resistance gene and providing evidence for a previously unrecognized mechanism of antibiotic inactivation. Expression of *gar* in *Escherichia coli* resulted in an eight-fold increase in gentamicin minimum inhibitory concentration compared to the empty vector control, confirming its role in resistance.

Biochemical analyses indicate that GAR modifies gentamicin through an ATP-dependent process. Liquid chromatography-mass spectrometry revealed depletion of the gentamicin substrate and the emergence of a new product with a mass consistent with phosphorylation (+80 Da). Formation of this product increased with enzyme concentration, supporting a catalytic mechanism. Parallel reactions with a known aminoglycoside phosphotransferase yielded a similar modified species, further supporting kinase activity. This conclusion was independently validated using a coupled pyruvate kinase/lactate dehydrogenase assay, which demonstrated ATP turnover during the reaction.

Structural insights from AlphaFold prediction revealed conserved features consistent with nucleotide-binding proteins, including a Walker A (P-loop) motif and putative metal-coordinating DxD motifs. Notably, GAR lacks significant sequence or structural homology to established aminoglycoside-modifying enzyme families, suggesting it represents a novel class of antibiotic-inactivating kinases with a distinct evolutionary origin.

A key experimental challenge was the limited solubility of GAR during heterologous expression. Despite extensive optimization of temperature, induction conditions, and expression systems, the protein was predominantly insoluble. However, a small soluble fraction was successfully purified and confirmed, enabling initial biochemical characterization.

Together, these findings establish GAR as a member of a previously uncharacterized family of kinase-like aminoglycoside resistance enzymes capable of phosphorylating gentamicin. This work expands the known diversity of resistance mechanisms and underscores the limitations of homology-based annotation in detecting novel ARGs. Continued characterization of GAR and related enzymes will be essential for understanding their biochemical properties and potential impact on antibiotic efficacy, informing future surveillance and therapeutic development strategies.

References:

1. Bohm, M. E., Razavi, M., Marathe, N. P., Flach, C. F., and Larsson, D. G. J. (2020) Discovery of a novel integron-borne aminoglycoside resistance gene present in clinical pathogens by screening environmental bacterial communities Microbiome 8, 41

Gen abstract type Poster

Section Type AEM

133 - Identification of commensal *Escherichia coli* isolated from healthy chickens that can Inhibit the growth of Avian Pathogenic *E. coli* in vitro

Zoé Cros[1], Jennifer Ronholm[1], Jinha Suh[1], Zhixuan Feng[1]

[1]McGill

Avian pathogenic *E. coli* (APEC) is a bacterium which can cause colibacillosis in poultry, a disease presenting as localized or systemic infections that can progress to fatal septicemia. This disease results in major productivity and economic losses worldwide. Traditionally, controlling APEC relied on antibiotics; however, growing concerns surrounding antimicrobial resistance (AMR) have accelerated the search for effective alternatives. Probiotics represent a promising approach to limit intestinal colonization by pathogens, primarily through competitive exclusion. However, no current probiotic formulation specifically targets APEC. One of the main challenges for this approach is the ability for probiotics to persist in their niche. The chicken gut microbiota presents a source of niche-adapted probiotic candidates. In this study, we aimed to identify and characterize commensal *E. coli* from healthy domesticated chickens that effectively antagonized the growth of APEC in co-culture, with the ultimate goal of developing a targeted probiotic cocktail to reduce APEC gut colonization in farmed chickens. To assess inhibitory activity, commensal *E. coli* isolates were co-cultured with a fluorescently tagged APEC strain, with APEC fluorescence serving as a proxy for growth suppression. Preliminary results validate this approach: a standard curve experiment demonstrated a strong linear relationship between CFU/mL and relative fluorescence units at 41°C (chicken internal temperature), with $R^2 = 0.96$, confirming that fluorescence reliably reflects APEC growth under physiologically relevant conditions. Of the 42 commensal *E. coli* screened, 17 significantly reduced APEC growth in vitro, with reductions of 25% or more. Selected inhibitory isolates will undergo downstream characterization of inhibition mechanisms and antimicrobial metabolite production, with the goal of identifying the most promising candidates for a defined, multi-strain probiotic cocktail with specific anti-APEC capacities.

Gen abstract type

Poster

Section Type

AEM

143 - Black Ice vs. White Ice: How Lake Ice Phenology Shapes Bacterial Community Structure

Alex Abhaya Wickramasinghe[1, 2], Pénélope Blackburn-Desbiens[2, 3], Mukund Gauthankar[2, 3], Milla Rautio[2, 3], Catherine Girard[1, 2]

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Over 86% of the world's lakes are covered by ice for at least part of the year and can act as a reservoir of microbial cells that are released into the water column during melt. However, climate warming shortens ice duration, delays its formation and accelerates its melting. Therefore, understanding the factors structuring microbial life in lake ice has become increasingly urgent. Ice properties differ markedly between Arctic and boreal regions, but it remains unclear how these differences in ice phenology shape bacterial communities in ice. Arctic lake ice typically forms thick, clear black ice that persists for most of the year, whereas boreal lake ice is thinner, shorter-lived and mainly composed of white ice largely formed from precipitation. Here, we present an amplicon-based survey of bacterial communities in lake ice and underlaying water across six Arctic and three boreal lakes spanning contrasting ice-formation processes, seasonal duration and watershed type. DNA extracted from ice and water samples was sequenced for the 16S rRNA gene. Bacterial community assemblages differed between boreal and Arctic lake ice ($p<0,001$, $r2=0,18$). Phycisphareae, Planctomycetes and Verrucomicrobiia dominated lake ice in the Arctic, but the latter were largely restricted to under-ice water in boreal lakes, possibly explained by regional landscape features and ice phenologies. On the other hand, Gammaproteobacteria and Bacteroidia were found more abundant in boreal than in Arctic lakes. Overall, species richness was similar between Arctic and Boreal lakes ($p=0,54$). These findings provide baseline insight into how ongoing changes in ice formation and duration may restructure microbial communities in northern lakes as warming accelerates ice loss.

Gen abstract type

Poster

Section Type

AEM

145 - Investigation of Metal Resistant Bacteria from Acid Mine Drainage Environments and the Genetic Elements Responsible

Gianna Kraus[1], Evan Guiney[1], Chris Maltman[1]

[1]Slippery Rock University

Microbe-metal interactions are an area of great interest in microbiology. Bacteria which possess a high-level of resistance to various toxic metals have the potential to provide us with mechanisms of remediating metal contaminated locations in an ecologically friendly way. That being said, to achieve this, one needs to have a better understanding of the genetic elements which provide this resistance, and we need to properly classify bacteria with these abilities. To this end, we investigated the genomes of 58 bacteria with a high-level resistance to one of 13 metals (Ag, Al, As, Au, Co, Cr, Cu, Mn, Ni, Pb, Se, Te, and Zn) isolated from environments impacted by acid mine drainage. Annotated genomes were screened for the presence of known metal tolerance/resistance genes related to these specific metals. Investigation showed these bacterial isolates do contain several various metal resistance genes and/or resistance mechanisms. Genes for resistance to some metals (Al, As, Co, Cu, Mn, Ni, Te, and Zn) were very common, appearing in the majority of isolates regardless of the metal they were initially selected against. Also, some metals didn't appear to have any known genes related to the metal they were selected against (Au and Se). Genetic elements for general heavy metal resistance were also identified, suggesting perhaps they are responsible for the tolerance observed as well. Methods of resistance appear to be similar across different species, suggesting a potential common evolution of these genes among distant bacterial lineages. The findings of this study are a significant advancement in the field of microbe-metal interactions and help further our understanding of the genetic elements involved in allowing bacteria to tolerate high levels of toxic metals and may highlight potential new genes responsible for this ability.

Gen abstract type

Poster

Section Type

AEM

147 - Bacterial Diversity in a BioCAST Reactor for Treatment of Gold Mining Wastewater

Juliana Alvarado-Drosou[1], David Walsh[1], Catherine Mulligan[2]

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Gold mining is one of Canada's most valuable industries. Industrial processes required in gold mining generate ammonia which contaminates mining waste streams. To prevent such waste streams from contaminating water bodies, a pilot-scale single-stage bioreactor called BioCAST was engineered to utilize partial nitrification and anammox processes to treat the gold mining wastewater. The unique, nitrogen-focused, chemolithoautotrophic environment within BioCAST is needed to support the growth of bacteria that facilitate the desired processes. However, it also selects for, and supports, an intriguing diversity of other microorganisms. Previously, 16S rRNA gene sequencing techniques were used to monitor the microbial populations contained in the bioreactor. Results identified key nitrifiers necessary for the remediation of the water *(Nitrosomonas* and *Nitrosospira*). Unexpectedly, a desired anammox species *Brocadia anammoxidans* was not directly observed while other anammox species belonging to the genus *Candidatus Brocadia* and *Candidatus Kuenenia* were abundant. The results implied that ammonia oxidation and anaerobic ammonia oxidation, processes required for the treatment of the wastewater, were likely occurring in the system through the bacteria as desired. Given that the BioCAST bioreactor has been in continuous operation for the last decade, several avenues for further investigation were made clear. Primarily, investigating the long-term evolution of microbes in this environment as a result of the persistent environmental pressures of BioCAST. Such selection pressures may drive the development of metabolic interdependencies and promote niche specialization to reinforce the functional stability of the nitrogen cycling in this distinct, artificial environment. Following that line of reasoning, long-read sequencing approaches were employed to assemble and characterize the genomes of microbes present in the bioreactor. Preliminary results reveal the genomes of numerous species of key nitrogen-cycling taxa were recovered as well as genomes belonging to additional phyla (Bacteroidota, Chloroflexota, etc.) thought to be metabolically linked with key taxa. Such results also highlight additional avenues worthy of future research, such as the impact of intracellular parasites and bacteriophage on these bacteria and their genomes.

Gen abstract type

Poster

Section Type

AEM

150 - Colorimetric Detection of *E. coli* Using a Fab-Based Nanoparticle Immunodetection Platform for Rapid and Sensitive Monitoring in Water Systems

Minghui Ma[1], Marc Aucoin[1], Bill Anderson[1], Shazia Tanvir[2], Amandeep Kaur[1, 2], George Botos[2], Trevor C. Charles[1]

[1]University of Waterloo, [2]ExactBlue

The availability of clean, safe water is essential for public health. However, contamination of water sources by pathogens such as *E. coli*, causing more than 2.2 million deaths per year, significantly contributes to the global disease burden. Millions of people, particularly in developing regions, are threatened by limited access to safe water sources, underscoring the urgent need for rapid, sensitive, user-friendly, and cost-effective pathogen detection applications for water surveillance. Current detection methods primarily rely on culture-based methods, molecular methods such as PCR, or immunological methods, such as ELISA. While these techniques are widely used, their application in routine monitoring is restricted by their inherent constraints: culture-based methods are time-consuming and therefore inadequate for rapid detection, while molecular methods and other novel biosensors are often not cost-effective, are equipment-dependent, or limited in sensitivity.

To address these challenges, this project proposed a nanoparticle-based immunodetection system employing two types of nanoparticles: relatively small antibody-conjugated magnetic nanoparticles, which are capable of capturing and concentrating the target cells (immunoseparation), and relatively large silica nanoparticles co-conjugated with beta-galactosidases and antibodies in separate layers, which serve as reporter particles (immunodetection). This system will quantitatively detect specific pathogens by generating measurable colorimetric signals within a short time frame when both nanoparticles simultaneously bind the pathogen in a sandwich configuration and are captured by a magnetic field. To date, we have successfully developed a functional nanoparticle detection system that integrates immunomagnetic separation and immunodetection, enabling the detection of approximately 100 cells within 45 minutes. The Fab fragment (V_H-C_{H1} and V_L-C_L) of a tested anti-*E.coli* O157:H7 antibody was sequenced and engineered for expression in *the E. coli* SHuffle strain. The use of expressed Fab significantly reduces the cost associated with intact antibodies for nanoparticle functionalization and enables a more flexible and efficient detection platform for pathogen surveillance. In future work, the optimized system will be benchmarked to validate its performance, sensitivity, and practicality for real-world applications. This user-friendly detection method has limited equipment requirements, such as a simple magnetic field and a spectrometer, making it readily applicable in diverse locations, especially where facilities are limited. In the long term, this novel approach is not limited to the specific pathogen selected in this project, as it can be adapted to detect multiple pathogens by modifying the antibody cocktail.

In conclusion, this project will advance waterborne pathogen surveillance by generating an applied quantitative and cost-effective waterborne pathogen detection tool that bridges the gap between traditional culture-based methods and modern molecular techniques, being as sensitive as culture-based methods (<10 CFU/mL) and as rapid as advanced detectors (within 4 hours) without the dependence on any advanced equipment.

Gen abstract type

Poster

Section Type

AEM

151 - Beyond ACC Deaminase: The Role of Bacterial Growth on ACC Rather Than ACCD Activity Alone in Shaping Plant Fitness

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Plant growth-promoting bacteria offer a sustainable alternative to chemical fertilizers, but their field performance remains inconsistent due to limited understanding of key traits under soil conditions. In particular, ACCD-producing bacteria (1-aminocyclopropane-1-carboxylate deaminase; encoded by *acdS*) reduce stress-induced ethylene levels in plants and thereby promote plant fitness and growth, by converting the ethylene precursor, ACC, to ammonia and α-ketobutyrate. While the core gene, *acdS,* has been well studied, little is known about the role of downstream genes that may be co-regulated with or functionally linked to *acdS* expression and end-product metabolism, or their role in plant stress mitigation.

Phylogenetic analysis of the native *acdS* operon in *Pseudomonas fluorescens* 8R6 (with experimentally confirmed ACCD activity), revealed multiple hypothetical proteins of unknown function (BLASTp, InterProScan, HMMScan, and MembraneFold) as well as two components (E1 and E2) of a pyruvate dehydrogenase-like enzyme. Sequence alignment followed by phylogenetic tree construction of the E1 components revealed a distinct cluster separate from pyruvate dehydrogenases (SwissProt), with 63.6% amino acid sequence identity to α-ketobutyrate dehydrogenase (BLASTp) within the same cluster suggesting α-ketobutyrate as the likely substrate and supporting a functional role of the entire operon in bacterial metabolism.

Thus, we generated *acdS* operon deletion mutants of *Pseudomonas fluorescens* 8R6 as well as derivative strains of *Pseudomonas fluorescens* 63-28 (harbors putative *acdS*, lacks ACCD activity) carrying stepwise insertions of an 8R6-derived *acdS* operon fragment, producing variants with progressively larger inserts, each incorporating an additional gene. Using these constructs, we investigated both the ability of bacterial growth on ACC as a sole carbon and/or nitrogen source and the (co-)expression patterns of the downstream genes as a single functional unit. By inoculating pak choi (*Brassica rapa* subsp. c*hinensis*) with these constructs, we further assessed the contribution of downstream genes to plant fitness (analyzing plant growth traits, chlorophyll content and ACC levels) under stress conditions and rhizosphere colonization. Overall, our work will help clarify whether plant benefits depend on coordinated *acdS* operon activity or ACCD activity alone, thereby informing the future development of more effective microbial inoculants.

Gen abstract type

Poster

Section Type

AEM

153 - Vertical stratification of mine tailings microbial communities along geochemical gradients one decade after organic cover application

Asma Asemaninejad[1, 2, 3], Graeme Spiers[2], Peter Beckett[2], Nadia Mykytczuk[2], Nathan Basiliko[3]

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Organic amendments that consume oxygen can function as reactive barriers, limiting sulfide mineral oxidation in acid-generating tailings; however, they may also promote metal mobilization in surface-oxidized horizons. One decade after applying a 1-meter thick organic cover, we observed notable yet stratified biogeochemical and microbial transformations down the reclaimed Anthroposol profile. The surface organic cover layer showed increased nutrient availability alongside declines in organic matter and C:N ratio, reflecting ongoing mineralization and decomposition. Beneath this layer, the interface zone exhibited microbial and geochemical convergence toward more soil-like conditions. Deeper tailings were slightly acidified, enriched in sulfur- and iron-oxidizing microbial taxa, potentially indicating continued sulfide weathering. Despite the improvements in the interface layer, switchgrass roots largely remained confined to the organic cover, hinting at possible chemical or physical barriers limiting deeper rooting. These findings highlight the short-to-medium-term success of organic covers in enhancing nutrient cycling and fostering microbial succession near the surface. However, persistent weathering underscores the need for complementary strategies, such as deeper amendments or reactive barriers. Given projected climate warming could accelerate organic matter decomposition and sulfide oxidation, increasing the risk of acid generation over time, these findings underscore the importance of sustained organic inputs and adaptive management to ensure long-term reclamation success.

Gen abstract type

Poster

Section Type

AEM

154 - Investigating Methanol Metabolism Bottlenecks and the Effects of Carbon Dioxide on Alphaproteobacterial Methanotrophs

Liliana Van[1], Lisa Stein[1], Dominic Sauvageau[1]

[1]University of Alberta

Methane emissions are a crucial topic of concern within the scope of the climate crisis. As the second most abundant greenhouse gas, methane has a global warming potential of 27-30 over 100 years when compared to carbon dioxide (USEPA, 2025). As such, innovative strategies are required in order for the most detrimental consequences of climate change to be mitigated. One popular strategy is utilizing methanotrophs, a diverse group of bacteria that utilize single carbon compounds as their sole energy source, such as methane and methanol. Alphaproteobacteria methanotrophs exhibit poorer growth on methanol compared to other aerobic methanotrophs as they may accumulate high concentrations of formaldehyde, a toxic metabolite, when cultured on methanol. Unlike Gammaproteobacteria, alphaproteobacterial methanotrophs can also utilize carbon dioxide. In this work, three alphaproteobacterial methanotrophs were grown under methane or methanol as primary carbon sources, and supplemented with carbon dioxide. Their growth was compared across treatment conditions, and assessed for extracellular concentrations of formaldehyde. *Methylosinus trichosporium* OB3b was the sole strain of the three that significantly improved in growth on methanol when carbon dioxide was added. In addition, all three strains accumulated high concentrations of formaldehyde when methanol was used as the carbon source, but no formaldehyde was detected when *Methylosinus trichosporium* OB3b was cultured with carbon dioxide and methanol. Transcriptomic analysis of *Methylosinus trichosporium* OB3b showed differential expression of genes in the serine cycle, carbonic anhydrase, and the glycine cleavage system that are presumably used to alleviate the bottleneck for formaldehyde oxidation and assimilation when growing on methanol and carbon dioxide.

Gen abstract type

Poster

Section Type

AEM

157 - Identifying Public Health Risks from Viruses Released in Glacial Melt

Harland Ammentorp[1], Andrea Silverj[1, 2], Tamara Rivera[3], Kristin Nielsen[3], Benjamin Barst[1, 4], Ann C. Gregory[1, 2]

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Accelerating climate change is driving rapid glacial retreat across Arctic and sub-Arctic regions, fundamentally altering microbial ecology in aquatic systems. Glacial ice represents a distinct microbial biome that harbors diverse and largely uncharacterized microbial and viral communities preserved within ice. As glaciers melt, these communities may be released into freshwater and marine ecosystems, potentially introducing novel viruses, microbial taxa, and functional genes into contemporary environments. This process raises important ecological and public health questions, particularly regarding the transport of virulence factors, antimicrobial resistance genes, biosynthetic gene clusters, and toxin associated elements via viral-mediated horizontal gene transfer.

This study investigates viral diversity, microbial transport, and potential ecological and public health risks along a glacial-to-marine gradient in Kachemak Bay, Alaska, a sub-Arctic fjord experiencing rapid glacial retreat. To capture a comprehensive glacial-to-marine gradient, field sampling was conducted across three watersheds with varying levels of glacial influence: Grewingk (heavily glaciated), Wosnesenski (moderately glaciated), and Jakolof Creek (non-glacial). Within each system, we collected viral and microbial samples from upstream sites and downstream estuarine zones to assess connectivity across tidal boundaries. By analyzing sediments, water, and filter-feeding mussels, this research provides a rare look at food-web integration in glacier-fed systems, which remain largely unstudied in North America outside of Greenland.

Samples are currently undergoing sequencing. In parallel, we are reanalyzing publicly available metagenomic datasets from glacier-fed streams. We have also compiled microbial and viral genomes from these glacier-fed stream datasets, along with Arctic marine systems and temperate and tropical marine environments as comparative benchmarks across multiple sites (n = 9,098 bacterial genomes; n = 499,507 viral genomes).

These preliminary analyses focus on identifying virulence factors, antimicrobial resistance genes, biosynthetic gene clusters, and toxin-associated elements linked to viral-mediated horizontal gene transfer, features that have not been compared across these ecosystems. Our initial results reveal that glacier-fed stream systems harbor distinct viral populations and microbial communities, with biosynthetic gene clusters dominated by terpenes and type III polyketide synthases, suggesting a high prevalence of defense genes. These features are observed across multiple glaciological environments, indicating both globally distributed functional traits and potentially unique signatures in Alaskan systems. Viral population clustering further demonstrates the presence of distinct glacier-fed stream populations, while also providing evidence that these populations are being transported into downstream marine ecosystems.

Once sequencing is complete, we will integrate our dataset with global glacier-fed stream systems to determine how our sites compare and to better understand how glacial meltwaters may act as conduits for microbial dispersal and functional gene transfer at a global scale. This work will provide new insight into the role of viruses in cryosphere-linked ecosystems and improve our ability to predict microbial and public health impacts in a warming world.

Gen abstract type Poster

Section Type AEM

158 - Dynamics of Microbial Separation: Overcoming Fouling Constraints in Microfiltration

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Microbial protein, also known as single-cell protein (SCP), is a promising alternative protein source for animal feed. It is produced from microorganisms such as bacteria, fungi, and yeast through fermentation. Compared with traditional protein sources such as soybean and fish meal, SCP production requires less land and water and has a lower environmental impact. However, this fermentation-based cellular agriculture requires environmentally sustainable and efficient cell harvesting techniques. Crossflow microfiltration could be a promising method for the aforementioned process due to its simplicity, environmental friendliness, high product selectivity, cost-effectiveness, and the absence of chemical additives. However, it is not without limitations. Membrane fouling and concentration polarization remain key challenges in such membrane separation systems. The formation and properties of those membrane fouling layers are strongly influenced by interactions between cells and the membrane, as well as among cells in the deposited layer. Microbial characteristics such as morphology, extracellular polymeric substance production, and deformation ability also influence fouling behaviors. Several methods have been developed to reduce the problem, including physical, chemical, biological, and hydrodynamic approaches. Hydrodynamic methods, such as membrane module vibration or rotation, increase shear stress near the membrane surface. Increasing shear rates near the membrane surface promotes cell back-transport, reduces concentration polarization, and limits cake layer formation. However, they require significant energy to operate, which increases processing costs. This study explored a simpler and more energy-efficient solution to improve the separation of *Methylophilus methylotrophus* from fermentation broth using crossflow filtration. The idea was to adjust the filtration chamber to control flow conditions without using additional energy or moving parts. To test this, a thin flat plate (shim) was inserted into the chamber to reduce its thickness by 0.63 mm. Filtration experiments were conducted under identical conditions, with and without the shim. Key parameters, including permeate volume, turbidity, and biomass concentration, were measured. The ability of microorganisms to deform and pass through membrane pores smaller than their nominal size has been reported to compromise cell retention during some shear-induced filtration processes. Especially gram-negative bacteria, which possess thinner and more elastic cell walls, tend to be more deformable and therefore more likely to penetrate small pores. However, this system achieved a 58 ± 4% increase in average permeate flux without affecting cell retention. As a result, the cell concentration has increased from 16.2 ± 0.5 g/L to 56.34 ± 0.08 g/L within two hours, compared to 35.44 ± 0.06 g/L in the control. Overall, this approach significantly improved harvesting efficiency, achieving a twofold increase, and provides an effective and low-cost alternative to traditional hydrodynamic methods while maintaining separation performance.

Keywords: Animal feed, Single cell protein; Downstream processing; Crossflow filtration; Microfiltration

Gen abstract type

Poster

Section Type

AEM

159 - Adaptive evolution of Methylomicrobium album BG8 for increased tolerance to acidic conditions

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Methane is a potent greenhouse gas with 28 times the warming potential of carbon dioxide over a 100-year timescale. The methanotroph *Methylomicrobium album* BG8 offers a potential mitigation strategy by using methane as its sole carbon source and converting it into high-value products such as biopolymers and biofuels. However, industrial applications of *M. album* BG8 remain challenging due to harsh conditions such as low pH, which imposes metabolic strain, slow growth and decreased product yields. Overcoming this limitation is critical to making methane bioconversion viable at scale. Thus, the goal of this research is to develop a strain of *M. album* BG8 that can thrive in low-pH conditions using adaptive evolution. Initially, cultures were grown in standard conditions with a pH 6.8 phosphate buffer before gradually increasing acidic conditions by lowering the buffer pH by 0.2 units after at least 5 passages. This process produced a strain of *M. album* BG8 capable of growing in pH 4.0 citric acid-phosphate buffer. When returned to neutral conditions then subsequently re-exposed to low-pH conditions, the adapted strain maintained comparable growth, indicating stable adaptation. To elucidate the adaptation mechanisms, Illumina NovaSeq whole-genome and RNA sequencing were conducted, as well as whole cell metabolomics analysis. The genome of the adapted strain was assembled against that of the parental, identifying key SNPs relating to acid tolerance mechanisms, including loss of S-layer production. The metabolic profiles of the adapted strain under low-pH and neutral conditions showed significant upregulation in the arginine biosynthesis pathway, suggesting ammonia production may be a predominant acid tolerance mechanism employed by the adapted strain. This is supported by a notable increase in pH of the culture during growth at lower pHs as well as colourimetric ammonia assay results. By overcoming the pH limitation that hinders industrial scalability, this research optimizes a strain of *M. album* BG8 for methane-based bioproduction while simultaneously providing insights into the molecular strategies methanotrophs use to adapt to acid stress.

Gen abstract type

Poster

Section Type

AEM

161 - Exploring plastic-associated biofilms on litter from Ontario lakes

Mia Rondinelli[1], Oona Esme[1], Aidan Vint[1], Ameneh Norouzian Ghahfarokhi[1], Tia Harrison[1], George diCenzo[1, 2]

[1]Queen's University, Department of Biology, [2]Queen's University, Department of Chemical Engineering

Amid mounting investigations into greener recycling alternatives, there has been a growing interest in understanding the composite populations of plastispheres. These are diverse anthropogenic environments where organisms live and evolve alongside plastic contamination and have been the source of a number of plastic-interacting microorganisms. Biofilm-associated microbes are of particular interest, since presumably the first step of microbial enzymatic degradation for many polymers is biofilm formation. Here, in collaboration with the Pollution Probe initiative Great Lakes Plastic Cleanup, we acquired small (generally <1cm^3) plastic debris samples from six Seabins, three docked in Lake Ontario (Hamilton & Cobourg) and three in Lake Simcoe (Barrie). Planktonic microbes were rinsed away, and a warm protease rinse was used to lift biofilms from the surfaces of the plastic debris. Aliquots of the resulting microbial suspensions were grown on several low-nutrient media to maximize captured diversity, and bacterial isolates were streak-purified from these “primary plates” and catalogued into our Open Plastic strain collection. This colony picking resulted in a library of 136 bacterial isolates. Then, DNA was purified from the remaining microbial suspensions, primary plates, and each individual isolate, and sequenced. Preliminary analysis revealed that the individual isolates span 19 different genera, though the majority of isolates were classified as *Aeromonas, Pseudomonas, Acinetobacter,* or *Exiguobacterium*. In future work, comparing the whole-genome sequences of these individual isolates to the biofilm metagenomes will allow us to understand the original biofilm compositions and assess which members of these populations were culturable with our methods. Comparing isolates to primary plate metagenomes will allow us to determine the proportion of culturable isolates we captured with selection and streak-purification. Finally, to assess the ability of individual isolates to grow with plastic as a sole carbon source, we are conducting a redox dye-based colorimetric growth assay in liquid minimal media with suspended pure polyethylene, polystyrene, polypropylene, and polyvinyl chloride microplastics. The first round of growth assays generated a shortlist of 40 strains which grew more efficiently on various microplastics than in negative controls where none were provided. Shortlisted isolates will be re-screened in colorimetric assays, then subject to proteomic analysis to see if they have the requisite enzyme pathways to interact with plastics.

Gen abstract type

Poster

Section Type

AEM

162 - Assessing the role of salinity on the growth and adaptation of soil microbial species and its effect on evolutionary trade-offs.

Julia Tuccitto[1], Lauren Hemara[1], Terrence Bell[1]

[1]University of Toronto

The soil microbiome composition is easily impacted by both naturally occurring and anthropogenic stressors, exerting negative impacts on the microorganisms present. This project aims to assess how salinity impacts the growth and survival of two soil isolates of interest from the Canadian Collection of Agricultural Soil Microbes - *Bacillus bombysepticus* and *Pseudomonas* sp. In particular, we are interested in understanding whether microbes have different adaptive responses across a stressor gradient i.e., at different salt concentration intensities. In this research, we inoculated these two isolates in a gradient of salt concentrations, no salt, 2%, 4%, and 8% salt, and measured their in-culture growth across 18 culture transfers (approx. every 3-4 days). After repeated transfers, we have determined these evolved lineages have adapted to the saline environment following successive passaging. We determined that regardless of stressor intensity, there were no negative trade-offs in growth or survival in the microbe's original environment, without additive NaCl. Additionally, we characterized phenotypic and functional differences in evolved cultures by visualizing cell size differences through microscopy, assessing in-soil growth with a microbial respiration assay, and assessing changes to protective and survival functions with a biofilm production assay. We identified no difference in biofilm formation, and no translatable adaptations to growth in saline soil after evolution to saline media. On the other hand, evolution to the saline stressor resulted in differences in cell size visualized through microscopy. Overall, we identified that the ability of microbes to adapt to stressors across a gradient in a simple media environment may allow us to predict how microbes respond to salinity in natural environments. Additionally, we identified that adaptation to a saline media environment does not result in functional differences in the assays we tested, nor negative evolutionary trade-offs in these isolates.

Gen abstract type

Poster

Section Type

AEM

163 - Evolving acid tolerance in *Methylobacterium extorquens* AM1 for improved industrial affinity

Maryssa Iacobelli[1], Dominic Sauvageau[1], Lisa Y. Stein[1]

[1]University of Alberta

Bioprocessing is a sustainable method for producing various compounds of economic value using living organisms. Industrial application of the bacterium *Methylobacterium extorquens* AM1 is of interest due to its ability to consume reduced single carbon compounds such as methanol - a common industrial waste product. However, the use of *Methylobacterium* in industry is limited by harsh conditions such as low pH, which can decrease product yield and growth, making the process overall inefficient. Therefore, the goal of this research is to develop an industrialized strain of *M. extorquens* AM1 capable of handling acid stress by using adaptive evolution. To adapt *M. extorquens* AM1, standard liquid culturing conditions for the strain were followed with the pH of the phosphate buffer being sequentially decreased by 0.2 every 5 passages. This allowed the strain to be brought down from growth at pH 6.8 to pH 6.2, when bioaggregates formed, and growth ceased. Thus, to continue adaptation, the cultures had to be supplemented with 1 mm glass beads to support initial cell adhesion and reestablish robust bacterial growth. This allowed the strain to be grown down to pH 5, before growth once again diminished. When the parental strain was grown at pH 5, little to no growth was observed, indicating adaptation. Stability of the adaptation was then tested by growing the strain back at pH 6.8 for 3 passages, before growing it back in media of pH 5 where unexpectedly lag phase was reduced. To characterize the new strain, DNA from liquid cultures of the parental and adapted strains were sent for Illumina NovaSeq whole genome sequencing. Returned reads were used to make a parental genome assembly using SPAdes with the ATCC provided *M. extorquens* AM1 genome used for mapping purposes. The assembly was quality confirmed with QUAST and annotated with Bakta, before being used to map pre-processed adapted genome reads and call for variants using Snippy. This analysis revealed 4 mutations of interest: a frameshift mutation in a potassium uptake protein, and 3 missense mutations in O-acetylhomoserine aminocarboxypropyltransferase, carbohydrate-selective porin OprB and CheY-like REC (which includes chemotaxis protein CheY and sporulation regulator Spo0F). Each of these mutations identify a potential way the adapted strain persists in acidic conditions. Further, these results demonstrate the potential role of pH in modulating extra polymeric substance production in *M. extorquens* AM1 and potentially other methylotrophs. To validate these results and further characterize the physiological basis for acid tolerance, transcriptomic and metabolomic analysis is being pursued. Overall, this research looks at improving the industrial affinity of *M. extorquens* AM1 by increasing its acid tolerance range, thereby supporting the development of more efficient bioprocesses.

Gen abstract type

Poster

Section Type

AEM

165 - A Coastal Arctic Ocean Microbial Time-Series Analysis

Sara Palestini[1], David Walsh[1], Dominique Baker[1]

[1]Concordia University, Department of Biology

The Arctic Ocean is warming at an accelerated rate and undergoing profound physical and biogeochemical change. Coastal regions are characterized by stratification of the water column, seasonal ice melt, freshening and dynamic nutrient gradients. These factors can strongly influence the microbial communities playing crucial roles in nutrient cycling, organic matter degradation, and form the base of the food web, making them key indicators of environmental change. Despite their essential role in ecosystem functioning, long-term microbial community responses to these environmental drivers remains poorly characterized. In this study, a decadal time-series of bacterial and archaeal community dynamics from the coastal Canadian Arctic Ocean is used to investigate its spatio-temporal variability. Community composition and activity are assessed using 16S rRNA marker gene and transcript sequencing and analysis, respectively. These data are integrated with environmental factors to identify key drivers of variability in microbial community structure. Emphasis is placed on vertical structuring of the water column, including comparisons between surface and subsurface chlorophyll maximum samples, a region where primary productivity is at its peak, as well as the influence of sea ice dynamics on community composition. Together, this work provides insights into the connection between environmental change and microbial community dynamics in a rapidly changing Arctic coastal system.

Gen abstract type

Poster

Section Type

AEM

167 - Characterization of Phage LF2, a New Polyvalent Phage Isolated on *Erwinia amylovora*

Logan Fehr[1], Jonathan Dennis[1]

[1]University of Alberta

Bacteriophages (phages) are a promising biocontrol agent with the potential to replace conventional antibiotic therapies for treatment of pathogenic bacteria. One commonly reported disadvantage of phage therapy is the high degree of host specificity, as the majority of phages can only infect members of a single host species. However, some phages may be characterized as being polyvalent and are able to infect more than one species of bacteria. Whereas polyvalency is considered relatively rare among phages, we have isolated a novel phage LF2 from wastewater that displays the ability to infect three distinct bacterial species belonging to the order *Enterobacterales*. The phage LF2 host range includes the phytopathogen *Erwinia amylovora* on which it was isolated, as well as two members of the *Enterobacterales* which are known human pathogens. Despite being isolated on a strain of *Erwinia amylovora*, the phage LF2 genome is far more closely related to other phage genomes previously isolated against other members of the *Enterobacterales*. The LF2 genome is 38,783 bp in size, has an average GC content of 51.0%, and has 42 predicted protein coding genes. LF2 is capable of suppressing the growth of all three susceptible species in liquid infection models, but was seen to be most active against *Erwinia amylovora*. Through the generation of LF2 spontaneously-resistant mutants, as well as via engineered deletion mutants, the putative receptor for phage LF2 was identified to be the LPS O-antigen in all three species. This suggests that among the phages which infect members of the *Enterobacterales*, similar receptor homologies may be facilitating a far greater degree of polyvalency than previously imagined. By further examining the binding site of phage LF2 in each species' LPS O-antigen, we anticipate a better understanding of all phage-host interactions in natural, heterogeneous populations of bacteria. Additionally, phages with broad-host-ranges represent an exciting solution to the deleterious issue of overly selective phages in robust phage therapy treatments.

Gen abstract type

Poster

Section Type

AEM

175 - Comparing the Efficiency of Three Soil DNA Extraction Kits

Keagan Waddington[1, 2], Pasindu Mudalige[1, 2], Srijak Bhatnagar[1, 2]

[1]Enviromics Research Group, [2]University of Calgary

DNA-based studies, such as those in microbial ecology, depend on high quality, bias-free DNA extraction to produce high quality results. While traditional phenol-chloroform extraction produces high molecular weight, high-yield DNA, the presence of contaminants and inhibitory chemicals can hamper the usability of extracted DNA downstream. Hence, DNA extraction kits are an effective way to obtain high quality, high purity DNA, with greater ease of use, and little to no toxic waste. But DNA extraction kits differ among themselves, from cost, to ease of use, to their extraction efficiency and DNA quality. Moreover, reliable and replicable DNA extraction is critical in microbial research, as it enables accurate species identification, better (meta)genome assembly, and functional annotation. This is even more crucial for environmental samples that often contain complex microbial communities with varying cell walls and inhibitory compounds that affect downstream DNA use.

Hence, this study evaluates the performance of three commercially available soil DNA extraction kits from three suppliers (QS, NS, and LS). These kits were selected based on manufacturing origin (Canada or not), cost, bead-based cell lysis combined with chemical disruption, as well as silica membrane-based DNA purification. DNA was isolated from a defined microbial community standard as well as from four types of environmental sample (soil, water, rhizoplane, and plastics). Kit performance was evaluated based on cost, ease of use, DNA yield, DNA purity, and microbial community representation. We found that Kit QS and Kit NS produced significantly higher DNA yields than Kit LS for standard community and soil, which are particularly relevant given these kits are optimized for soil DNA extraction. However, no significant differences in yield were observed among the kits for river water and rhizoplane samples, which suggest that sample type plays a key role in extraction efficiency. Additionally, all three kits yielded comparable quality of DNA, and all showed an elevated absorbance at 230 nm indicating potential presence of contaminants. Analysis of microbial community revealed notable differences in community representation. For the standard microbial community, Kit QS and Kit LS more accurately reflected expected bacterial relative abundances, Kit NS showed lower accuracy, particularly for Gram-positive bacteria, likely due to differences in cell lysis. Kit NS uses bead vortexing for cell disruption, while Kit QS and Kit LS use bead beating. So either the cell rupture was incomplete, or the prolonged vortexing sheered DNA from easy to lyse cells.

Overall, this study demonstrates that while all three kits do produce usable DNA, important differences exist in yield and community representation depending on the sample type and extraction method. These findings highlight the importance of selecting an appropriate DNA extraction kit to fit specific research needs, while simultaneously balancing cost, convenience, and performance.

Gen abstract type Poster

Section Type AEM

178 - Exploring genetic variation for mycelia-based materials

Jianping Xu[1], Viraj Whabi[1, 2]

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Fungal mycelia are attracting widespread attention as environmentally and economically sustainable alternatives to traditional plastics and textiles. The mycelia of different fungal species and strains can vary widely in their chemical compositions and in how the various chemical components are organized within and among cells, both with significant influences on materials properties of fungal mycelia. However, little is known how genetic differences among strains and species impact chemical compositions and materials properties of fungal mycelia. In this presentation, using the common split gill mushroom *Schizophyllum commune* as a model, we revealed abundant genetic variations within this species as well as environmental factors that impact mycelial materials properties. Our results demonstrate significant potential to genetically tune fungal mycelial materials.

Gen abstract type

Poster

Section Type

AEM

179 - A Novel In-Soil Assay Shows That Some Bacterial Biofertilizers Lack Essential Motility Capabilities

Aisha Chan[1]

[1]Department of Physical & Environmental Sciences, University of Toronto

The plant microbiome contains communities of microbes that can positively affect the plant's health. Biofertilizers that aim to extract these beneficial microbes for agricultural applications are largely unreliable, unable to form stable in-soil microbe populations. One underexplored aspect of bacterial biofertilizer strains are their in-soil motility, which may impact how well they can migrate from the topsoil to their ideal plant root microhabitat. So, for biofertilizer strains that are being applied to farm soil, their motility capabilities and in-soil dispersal may be an important determinant of their success. In the natural environment, bacteria can use flagella to swim in liquids and to swarm on soft surfaces. Assessing these movement abilities may give insight into how well they can disperse in-soil. As part of the Genome Canada-funded BENEFIT project, we developed two novel assays for screening the motility capability of potential biofertilizer strains. The in-culture swimming and swarming phenotypes of diverse bacterial taxa was assessed in addition to their in-soil motility. We hypothesize that the swimming and swarming abilities of the strains positively correlate with their in-soil dispersal capabilities, and that bacteria from different taxa have different motility capabilities. To measure the swimming and swarming capabilities of potential biofertilizers, the strains were inoculated into the center of a 0.3% swimming agar which has a liquid-like consistency, and a 0.5% swarming agar which has a soft surface. The agar was recorded with a GoPro to assess how fast they can spread to the edge of the plate after 60 hours. To measure bacterial in-soil dispersal abilities, strains were spiked into the center of a soil plate, left for 7 days and soil cores sampled at the center, edge, and intermediate distance of the plate for CFU counting. Results demonstrated that some candidate inoculants - such as *Ensifer* and *Dyadobacter* - are fast in-soil dispersers. Other inoculants with desirable plant-growth promoting traits (e.g., *Pseudomonas bijieensis* and *Streptomyces narbonensis*) demonstrate poor in-soil dispersal which may hamper their benefit following in-field application. This research has developed a valuable tool for assessing inoculant environmental compatibility and dispersal potential in the field. We also find that swimming and swarming speeds are for the most part, positively correlated with soil motility which makes it possible to use a straightforward swimming and swarming in-vivo assay as a proxy for potential in-soil success. Additionally, it was observed that biofilm formation in the swarming assays might play a factor in increasing bacteria swarming motility.

Gen abstract type

Poster

Section Type

AEM

183 - Microbial ecology of milk products and processing facilities

Allie Shiyuk[1], Jonathan Van Hamme[1], Eric Bottos[1]

[1]Thompson Rivers University

The presence of endospore-forming bacteria in milk products and processing environments poses a threat to the milk powder production industry. While conventional pasteurization and heat treatments throughout processing eliminate most bacteria, spore-formers can survive by producing highly resistant structures called endospores that can persist in these environments, threatening product quality, safety, and shelf-life. This research aimed to characterize spore-forming bacterial communities in milk processing facilities and their associated products using 16S rRNA gene amplicon and metagenomic sequencing approaches, while monitoring bacterial community dynamics throughout production.

Raw milk, milk protein concentrates, processing plant swab samples, and milk powder products were received from two milk production facilities in Canada. Bacterial community composition was assessed using 16S rRNA gene sequencing, and metagenomic sequencing was performed to explore the functional potential of microbial communities.

Based on 16S rRNA gene amplicon sequencing data, the core microbiome across milk and dairy samples consisted of Firmicutes (48.8%) and Proteobacteria (32.1%). Several low abundance taxa including Actinobacteroidota (7.6%), Deinococcota (5.7%), and Bacteroidota (4.0%) were also consistently observed. We found that sample type exerted a significant effect on bacterial community composition, explaining 26.4% of the variation in all amplicon sequence variants (ASVs) within samples, and 28.7% of the variation in ASVs linked to spore-formers. Among the endospore-forming bacteria detected, *Bacillus*, *Clostridium*, *Lysinibacillus*, *Robertmurraya*, and *Caldalkalibacillus* drove changes in community structure between sample types. Bacterial communities within raw milk, skim milk, milk powder, and processing plant swab samples were significantly influenced by collection date, highlighting changes in community structure over time. Finally, we sequenced and analyzed 90 metagenomes and used the Kyoto Encyclopedia of Genes and Genomes (KEGG) to characterize the functional profile of microorganisms present, revealing dominant functional pathways. Across all samples, the dominant KEGG level 1 functional categories included metabolism, cellular processes, and environmental information processing. At KEGG level 3, metabolism was represented by metabolic pathways (ko01100), biosynthesis of secondary metabolites (ko01110), and microbial metabolism in diverse environments (ko01120), while quorum sensing (ko02024), ABC transporters (ko02010), and two-component systems (ko02020) were abundant functional pathways within the cellular processes and environmental information processing categories.

Taken altogether, these data enrich our understanding of endospore-forming bacterial communities in milk powder production facilities and their associated products and will inform the development of a quantitative digital polymerase chain reaction (dPCR) assay for the rapid and sensitive detection of spore-formers in raw milk, processing intermediates and powdered products.

Gen abstract type Poster

Section Type AEM

184 - Comparative Metagenomic Analysis of Vulture Gut Microbiomes

Zakie Hage[1], Andrea Silverj[1, 2], Amanda Andrade[1, 2], Ann Gregory[1, 2]

[1]University of Calgary, Alberta, Canada, [2]Snyder Institute for Chronic Diseases, Alberta, Canada

Vultures are raptors that perform a critical ecological role as obligate scavengers cleaning up carcasses (carrion), an underappreciated role. Due to carrion being a hotspot for pathogenic bacterial growth and toxins, the vultures have evolved a protective gut microbiome that is tailored to feeding on carrion. Previous studies on the gut microbiome of the Himalayan, Turkey, and Black Vulture, along with the Andean and California condor have focused on the bacteria, missing the roles of viruses and eukaryotes in their defence.

In many organisms, the gut microbiome regulates nutrient absorption and metabolic homeostasis. In vultures, microbial communities are tailored to supporting their highly pathogenic carrion-based diet, with enrichment of taxa capable of host protection, protein degradation and nutrient recycling.

To better understand these protective functions, I reanalyzed five publicly available vulture metagenomic datasets to explore the multi-kingdom composition and metabolic capabilities of their gut microbiomes. Shotgun reads were assembled and binned to recover bacterial metagenome-assembled genomes (MAGs), and viral contigs were identified and clustered into vOTUs, followed by functional annotation of metabolic pathways and auxiliary metabolic genes (AMGs). In total, I found 72 high- and medium-quality MAGs, 13,955 vOTUs, and detected *Candida spp.* in a subset of samples. Across all vultures' species, *Fusobacterium* and *Clostridium* were consistently abundant, with genomic potential for protein degradation and the production short-chain fatty acids (SCFAs), supporting carrion digestion and modulation of host inflammation. These taxa also encoded biofilm-associated traits that may limit colonization by competing or pathogenic microbes. Among the viral populations, the majority of annotatable vOTUs were tailed phages within the class Caudoviricetes. In addition, 2,075 vOTUs were ssDNA viruses in the order Petitvirales, most of which originated from the sole dataset generated using multiple displacement amplification, a method known to bias the recovery of ssDNA viruses. Metabolic analyses are ongoing; however, preliminary results indicate the presence of pathways associated with SCFA production and other mechanisms linked to inflammation reduction.

My alpha diversity analyses revealed clear differences between the bacterial and viral communities within each species. The bacterial and viral richness varied significantly between host species. There was less variation in the bacteria compared to the viruses suggesting that there is a stronger association with the host. In contrast, the viromes higher diversity suggests that there is high inter individuality. These patterns were consistent with my beta-diversity analyses, where bacterial communities showed tighter clustering by host species (PERMANOVA R^2 = 0.336, p = 0.001), indicating a structure that is driven by the host. The virome was more dispersed (PERMANOVA R^2 = 0.229, p = 0.001), reflecting more inter-individual variability. Together, this explains that the bacterial microbiome is more stable and conserved based on host species, while the virome is more dynamic with inter-individual variation, likely driven by phage-host interactions and environment exposure from carrion. This pattern is consistent with observation in the humans.

Overall, these findings provide the evidence that vulture species have convergently evolved a gut microbiomes functions tailored to carrion digestion, pathogen defence, and inflammation reduction despite species-specific bacteriomes and viromes.

Gen abstract type Poster

Section Type AEM

185 - Monitoring putative desulfonation genes involved in PFAS degradation by *Gordonia* sp. NB4-1Y using quantitative dPCR assays

Elissa Ony[1], Sharon Brewer[1], Eric Bottos[1], Jon Van Hamme[1]

[1]Thompson Rivers Univesity

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are anthropogenic fluorinated compounds that are considered environmental contaminants of concern due to their extensive use, toxicity, and bioaccumulative potential. PFAS are characterized by long fluorinated carbon chains containing strong carbon-fluorine bonds, which promote chemical stability and resistance to biodegradation. The use of certain PFAS, including 6:2 fluorotelomer sulfonamidoalkyl betaine (6:2 FTAB) and 6:2 fluorotelomer sulfonate (6:2 FTSA), as ingredients in aqueous film-forming foams (AFFFs), has contributed to widespread environmental contamination. The aerobic soil bacterium *Gordonia* sp. strain NB4-1Y, a Gram-positive bacterium originally isolated from vermicompost, has been shown to degrade 6:2 FTAB and 6:2 FTSA to utilize them as sulfur sources for growth. In this study, quantitative chip digital polymerase chain reaction (dPCR) assays were designed for six genes of interest and two reference genes, which were validated using a combination of end-point PCR, Sanger sequencing, and quantitative PCR (qPCR). The genes of interest, which encode a dimethylsulfone monooxygenase (SnfG) (RS05625), three LLM class flavin dependent oxidoreductases (RS08775, RS08755, RS08670), a CrcB fluoride efflux transporter (RS20500), and an acyl-CoA dehydrogenase family protein (RS21130), were selected based on previous transcriptome and proteome data. Growth kinetics of *Gordonia* sp. strain NB4-1Y were analyzed in a time course experiment conducted in biological triplicate where NB4-1Y was provided with 6:2 FTAB, 6:2 FTSA, a non-fluorinated analog of 6:2 FTSA (1-octanesulfonate), or $MgSO_4$ as sole sulfur source. Optical density (OD_{600nm}) and fluoride ion release were monitored, and cell pellets were collected for RNA extractions at 13 time points over a period of 22 days. Validated quantitative PCR assays and chip digital PCR was used to quantify gene expression across treatments for six genes of interest and one reference gene at four time points, after 67, 160, 303, and 470 hours of incubation. One-way analysis of variance (ANOVA), followed by Tukey's Honest Significant Difference (HSD) post hoc test, was used to determine whether gene expression differed with sulfur source provided. We hypothesized higher expression for genes of interest when NB4-1Y was exposed to 6:2 FTSA or 6:2 FTAB compared to $MgSO_4$. Gene expression was found to shift between growth phases. Furthermore, sulfur source significantly affected expression of five genes of interest at 67 hours, with treatment with 6:2 FTSA exhibiting the highest gene expression. Expression of target genes was not affected by treatment at time point 6 (160 hrs) and expression of only one target (RS08670) was affected at time point 9 (303 hrs). Treatment also affected the expression of all gene targets at 470 hours with the no sulfur source + 50 % ethanol and 6:2 FTAB + $MgSO_4$ treatments exhibiting the highest expression levels. Comparisons of these data with transcriptome and proteome data will inform recommendations made to guide future protein purification and characterization experiments.

Gen abstract type Poster

Section Type AEM

186 - *Bacillus* endospore purification for enumeration and quantification

Janelle Li[1], Amelia Danzinger[1], Jayne Rattray[1, 2], Casey Hubert[1]

[1]University of Calgary, [2]Canadian Natural Resources Limited

Certain bacterial species within the Bacillota phylum are able to enter a dormant state and form endospores to contend with unfavourable conditions for growth. Endospores are highly resistant to a variety of harsh conditions that would otherwise kill growing (vegetative) cells. Primary methods for endospore enumeration involve the quantification of endospore biomarker dipicolinic acid (DPA), which can be converted to endospore count using a standard conversion factor of 2.24×10^{-16} mol spore^{-1}. However, this factor is not universally accurate for all spore forming species, varying especially among thermophilic endospore formers. In order to calculate the DPA levels in endospores and develop tailored DPA conversion factors, a robust method of spore purification and quantification is needed.

Freeze-thaw cycles, thermal inactivation, osmotic pressure, sonication, and density gradient centrifugation were applied to pure cultures of *Bacillus spizizenii* and *Bacillus cereus*, consisting of both vegetative cells and endospores. Preliminary tests show cell lysis by freeze-thaw cycles and thermal inactivation to be the least effective, with noticeable losses in vegetative cells but also endospores. Cell lysis by osmotic pressure was more effective, with noticeable but insufficient reductions in vegetative cells to be used as a stand-alone method. Purification by density gradient centrifugation was most effective, with minimal losses in endospores and significant reductions in vegetative cells. Water washing to induce osmotic stress, used in combination with centrifugation separation may further improve purity, as the initial killing of vegetative cells can decrease the number of cells to be removed from vegetative cell layers of the density gradient, preventing clogging and mixing with lower layers containing endospores. Centrifugation was more effective for endospores not known to have an exosporium. Species such as *B. cereus* have an exosporium that likely increases the natural aggregation of vegetative cells and spores, impacting the ability to separate the spores based on density. Ultrasonication prior to centrifugation of these species may separate aggregates and allow for effective separation based on density.

This research will allow for accurate endospore enumeration in future studies, and thus a greater understanding of the role and abundance of bacterial endospores in medical, industrial and environmental contexts.

Gen abstract type

Poster

Section Type

AEM

193 - Optimizing the time-to-result for lateral flow immunoassay-based detection of Enterohemorrhagic *E. coli* (EHEC) O157

Ananya Nanda[1], Ryan McNeice[1], Darius G. Rackus[1,2], Dustin J. Little[1]

[1]Toronto Metropolitan University, [2]Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Toronto, ON

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a significant human pathogen responsible for causing severe gastrointestinal disease. Animals serve as the natural reservoir of EHEC and are the primary source of transmission through consumption of raw or undercooked meat, unpasteurized dairy products, or via faecal contamination of agricultural produce during food processing and manufacturing. EHEC infections can cause severe complications, including haemolytic uremic syndrome (HUS) and renal failure, and conventional antimicrobial treatments promote the release of Shiga-like toxins that increase infection-associated mortality. Therefore, early detection and prevention of EHEC contamination in food manufacturing and processing pipelines is critical. Although several conventional methods exist for detecting EHEC in food samples, rapid, portable, and cost-effective diagnostic tools such as lateral flow immunoassays (LFAs) offer promising solutions. However, the need for sample pre-enrichment and long processing times (24-48 hours) of LFAs remains a major limitation for widespread and rapid use. Currently, two main LFAs are approved for use by the Government of Canada for microbiological analysis of foods to detect the EHEC O157 serotype, namely the SinglePath and VIP Gold devices. Both LFA devices rely on detecting the O157 antigen, which is a major part of the lipopolysaccharide (LPS) and group four capsule surface polysaccharides.

In this study, we aimed to improve the time-to-result of LFA devices to detect EHEC O157 by investigating EHEC growth dynamics in various pre-enrichment medias, determining the limit of detection (LOD) for laboratory-grown EHEC O157 strains, and enhancing signal intensity of the LFA devices using silver staining and automated imaging and quantification. Additionally, we investigated the ability of LFA devices to detect EHEC variants that express variable amounts of their O157 antigen in culture. Under these conditions, we determined that tryptic soy broth (TSB) with novobiocin induced expression of lipopolysaccharide in EHEC O157 in WT and LPS reduced variant strains, determined the LOD of EHEC O157 and LPS variant strains for the LFA devices, and developed a silver enhancement strategy in combination with an imaging platform to capture standardized and uniform images of the devices for quantitative analysis.

Overall, this study demonstrates that by optimizing pre-enrichment growth conditions and enhancing signal detection improved assay performance and time-to-result. Together, this data supports the development of rapid and sensitive diagnostic tools for food safety and public health applications.

Gen abstract type

Poster

Section Type

AEM

200 - Grassroots Restoration: Harnessing Plant Probiotics for Native Albertan Prairie

Pasindu Mudalige[1], Renzaj Aien Paguergan[2], Lawrence Park[2], Dale Pulvermacher[3], Jo Johnson[3], Rosa Martinez Lozano[3], Tyler Waldron[4], Emilie Brien[5], Alia Snively[5], Ayla Peacock[5], Marilyn Neville[5, 6], Adriana Morrell[3, 4], Srijak Bhatnagar[1, 2]

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Native prairie in Alberta is currently threatened by the conversion and fragmentation of wild landscapes due to agricultural and industrial development. Currently prairie restoration focuses on transplanting and seeding native grasses, such as fescues and wheatgrasses, onto disturbed sites. However, reestablishment is often challenging due to transplantation failures. One very likely reason is that traditional restoration practices do not account for the roles of plant microbial communities. Microbes associated with plants can improve nutrient uptake, confer drought and pathogen resistance, and act as indicators of ecosystem and host-plant health. Therefore, as native prairie continues to be threatened, it is imperative that we leverage microbes to restore native prairie grasslands.

Here, we study the effect of commercial microbial soil amendments (i.e., bacterial and/or mycorrhizal probiotics) on the epiphytic and endophytic root microbiomes (bacterial and fungal) of three native grasses, Rough Fescue (*F. campestris)*, Idaho Fescue (*F. Idahoensis)*, and Blue Bunch Wheatgrass (*P. Spicata)*. Seeds collected from wild plants of these species were germinated in potting mix and then inoculated with three probiotic treatments: 1) Dynomyco, 2) Mykos both of which containing only mycorrhizae, and 3) GreenGro a mycorrhizal and bacterial consortium, each in replicates. Three control conditions for each species were also created, in which germinated seedlings were transferred to unamended potting mix, pasteurized unamended potting mix, and an unamended native prairie soil mixture. Five months post-inoculation, three different root microbiome habitats were collected: rhizosphere, rhizoplane and endosphere.

From each sample, the V4 region of 16S RNA gene and the V7-V8 region of 18S rRNA gene were amplified and sequenced to assess bacterial and fungal communities respectively. Sequence QC and Amplicon sequence variant inference were conducted with DADA2, followed by diversity estimates and statistical testing using the Divnet and vegan R packages.

We confirm the root colonization of the applied microbial amendments. We also found arbuscular mycorrhizae (AMF) present in all but pasteurized control. Unsurprisingly, the AMF species was amendment-specific and differed from the native soil, which contained *Rhizophagus, Glomus, and Claroidoglomus*. The bacterial communities of the rhizosphere, rhizoplane, and endosphere, although sharing overlapping taxa, had statistically different community compositions, with alpha diversity increasing along the interior to exterior axis of the root. Importantly, the amendment was the stronger indicator of community composition than the plant species for the two fescues, except for native soil treatment. This work, to the best of our knowledge, is the first testing of commercial microbial amendments in native prairie grasses, and we are currently in the process of testing these probiotics in situ on prairie along the foothills of southern Alberta.

Gen abstract type Poster

Section Type AEM

202 - Phages in fungal soup: the influence of fungi on phage-bacterial interactions

Rabia Fatima[1], Alexander Hynes[1]

[1]McMaster University

Phages (bacterial viruses) are key drivers of bacterial community dynamics. However, the interaction between the phage and its bacterial host does not occur in isolation, but rather in complex communities consisting of other microbes, including eukaryotic ones such as fungi, which also compete for the same resources. As fungi are prolific producers of bioactive secondary metabolites, we sought to investigate whether fungal metabolites can directly influence phage infection to promote or interfere with growth of the bacteria. We screened phages infecting *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* in spent media generated from clinical strains of *Candida albicans,* an opportunistic human fungal pathogen. Most interactions we detected could be attributed to acidification of the medium and were not followed up on. However, fresh fungal medium (yeast peptone dextrose) inhibited infection of *E. coli* phage Mu, while spent medium of one *C. albicans* strain rescued this inhibition. Both the bacterial host and phage remained stable in the fresh and spent media, indicating the effect is specifically on phage infection. Substituting dextrose in the fungal medium with other carbon sources (fructose, galactose, glycerol, sucrose, and maltose) revealed that only fructose can fully recapitulate both the inhibition phenotype of fresh medium and rescue phenotype of the spent medium, hinting at a specific fungal response. Furthermore, adding dextrose back into the media revealed that phage inhibition and rescue are likely independent phenotypes, such that dextrose reduced phage titer in fresh medium, but the rescue phenotype was not observed when spiked into the spent medium. The phage infection rescue phenotype of fungal spent media was generalizable to other phages and bacterial hosts but remained phage-bacterium pairing specific. Protease treatment and heat denaturation of fungal spent media did not abolish the inhibition and rescue phenotype suggesting that the fungus is using a non-protein signal to influence phage infection. Overall, studying phage-bacterial dynamics in the context of other microbes, fungi specifically in this study, will improve our understanding of their interactions within complex natural environments.

Section Type AEM

Poster & Abstract selected for Symposium 12 - Fungi as Nature's Engineers for Health, Environment, and the Sustainable Bioeconomy June 18, 2026

203 - Synergistic microbial consortia and abiotic constraints: drivers of *Acer saccharum* growth during northern migration

Morgane Enea[1, 2, 3], Isabelle Laforest-Lapointe[1, 2, 3]

[1]Université de Sherbrooke, [2]Centre d'étude de la forêt, [3]Centre SÈVE

As climate change accelerates, the poleward migration of temperate tree species is influenced by soil microbial communities. While abiotic factors are traditionally prioritized in range-shift models, host-microbe symbioses represent a significant factor in forest resilience. This study investigates the sugar maple (*Acer saccharum*, Marshall), whose northern expansion into the boreal biome may be influenced by the microbial composition of novel northern soils. To test this, a controlled greenhouse experiment was conducted over an 8-month period. *Acer saccharum* seedlings were inoculated with living forest soils collected from natural altitudinal gradients at Mont Mégantic and Mont Écho, Quebec. To decouple the functional roles of specific microbial groups and evaluate the role of arbuscular mycorrhizal fungi (AMF), targeted antifungal and antibacterial treatments were applied to the forest inocula (7% of total pot volume). Microbial community assembly was monitored via 16S and ITS rDNA amplicon sequencing across different developmental stages. Growth trajectory monitoring revealed significant interactions between microbial treatments and time ($p<0.001$). Seedlings associated with the complete microbial community exhibited a substantial increase in performance, yielding an additional 29 cm in height compared to those in autoclaved soil. Notably, while the hypothesis focused on AMF as primary drivers, the targeted suppression of the bacterial community reduced this microbial growth benefit by approximately 18%. This suggests that AMF efficacy is enhanced by the associated bacterial consortium. Furthermore, seedlings grown in 100% forest soil demonstrated the lowest overall performance, likely indicating that abiotic stressors such as low pH or the presence of boreal pathobiomes act as barriers to sugar maple establishment. These findings indicate that AMF require synergistic microbial interactions to maximize host fitness during range expansion. These results highlight the importance of the complete microbial community in forest migration dynamics. Integrating multi-trophic interactions into predictive models is necessary to accurately assess the sustainability of temperate forests under global change.

Gen abstract type

Poster

Section Type

AEM

206 - Elucidating the Role of a Methanotroph-Specific Bacteriohemerythrin in Biofilm Formation

Nina Deschner[1], Cerrise Weiblen[1], Dominic Sauvageau[2], Lisa Stein[1]

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Microorganisms such as methane-oxidizing bacteria (MOB) offer a promising strategy to mitigate climate change due to their ability to consume the greenhouse gas methane (CH_4). *Methylomonas denitrificans* FJG1 is notable for its use of a methanotroph-specific bacteriohemerythrin homolog (*bhr-00*) to persist under hypoxic conditions. Recent studies further suggest a link between *bhr-00* expression and polysaccharide production, a major component of extracellular polymeric substances (EPS), implicating bacteriohemerythrin in biofilm formation. This study investigates the role of *bhr-00* in MOB biofilm formation and biofilm-mediated CH_4 sequestration. To address this, *Methylomicrobium album* BG8, which lacks native bacteriohemerythrin genes, was engineered to heterologously express *bhr-00*. Plasmid transfer was achieved via conjugation, and *bhr-00* expression was validated using Western blotting and UV-visible spectroscopy of purified protein. Using established methods, monoculture biofilms of *M. denitrificans* FJG1, *M. album* BG8, and engineered BG8 (*bhr-00*) were cultivated in methane-oxygen countergradient syringe hydrogels to assess polysaccharide production associated phenotypes. Wild-type BG8 exhibited a reduced capacity for polysaccharide production, a major component of EPS, in the hydrogel system compared to FJG1 and BG8 (*bhr-00*). To further evaluate the role of *bhr-00* in biofilm function, the syringe hydrogels were segmented into individual discs and methane oxidation was monitored in sealed vials via gas chromatography. Preliminary results indicate that wild-type BG8 forms attenuated biofilms and removes methane less efficiently than BG8 (*bhr-00*) and FJG1. Notably, FJG1 and BG8 (*bhr-00*) biofilms rate of methane oxidation was nearly double that of wild-type BG8 in the sealed vial system. Further fluorescent microscopy analysis is being pursued to characterize the architecture of hydrogel-based biofilms and clarify the relationship between *bhr-00* expression and productive biofilm formation in methanotrophs.

Gen abstract type

Poster

Section Type

AEM

207 - Berry Interesting: A Pan-Family Analysis of Ericaceae Rhizosphere Microbiomes

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Plants in the Ericaceae family, like blueberries (Vaccinium spp.) and Labrador Tea (R. groenlandicum), are commercially and culturally valuable for health and nutrition, including the production of antioxidant and anti-inflammatory compounds. Extensive research has explored the relationship between ericaceous plants and ericoid mycorrhiza, which enhance nutrient uptake and confer stress resistance, enabling these plants to thrive in nutrient-poor, acidic soils. Despite the profound research on ericoid mycorrhiza, the role of Ericaceae-associated bacterial communities remains comparatively understudied. Bacteria, much like fungi, are key contributors to plant health and stress tolerance. Given that Ericaceae species thrive in similar extreme soil conditions, they may share a conserved bacterial microbiome contributing to their ecological success. However, existing studies have largely focused on individual species, with no pan-family comparisons. Hence, we present a meta-analysis of the Ericaceae rhizosphere, comprising 11 studies across 11 species from the Ericaceae family.

Publicly available 16S rRNA gene amplicon datasets of rhizosphere communities spanning Ericaceae species were retrieved from the NCBI Sequence Read Archive and combined with in-house data from Labrador tea (11 datasets total). Datasets were grouped by variable region and processed individually to generate phylotype abundance tables. Sequence trimming and quality control were conducted using Cutadapt and DADA2. Reads were dereplicated (minimum redundancy of 5 reads), denoised, *de novo* chimera-checked, and clustered at 97% OTU similarity using VSEARCH. Taxonomy was assigned using the SILVA 16S database (v138.1) and aggregated at the genus level. Datasets were merged into a single phylotype table and normalized (minimum 5000 reads; CLR transformation) to account for differences in sequencing depth. Alpha and beta diversity were assessed using phyloseq, and the core microbiome was identified through prevalence- and abundance-based approaches.

The cumulative dataset had a mean and median sequencing depth of 39,156 and 32,931 reads per sample, respectively. Pairwise comparisons using Wilcoxon tests with Benjamini-Hochberg correction revealed significant differences in alpha diversity among host species. Differences were more pronounced for the inverse Simpson than for the Shannon index. This suggests that variation in community evenness and dominance structure contributed strongly to the observed patterns. Principal coordinates analysis ordination using Aitchison's distance showed that community composition varied significantly across host species, geographic location, and study.

To the best of our knowledge, this study provides the first pan-family analysis of the bacterial rhizosphere across various Ericaceae plants. We identified *Bryobacter*, *Sphingomonas*, and *Reyranella* as shared genera present in 10 of the 11 Ericaceae species. These genera are associated with acidic environments, nutrient and carbon cycling, alongside conferring stress tolerance. Thus, the presence of shared genera across diverse hosts and geographic regions suggests a conserved Ericaceae microbiome associated with adaptation to nutrient-poor, acidic environments. Although functional roles cannot be directly inferred from 16S data, these findings highlight candidate taxa for further investigation. Future studies using metagenomics or other functional approaches may clarify their roles and support applications in agriculture and conservation of culturally important Ericaceae species such as Labrador tea.

Gen abstract type Poster

Section Type AEM

213 - Exploring the Antibacterial Activity and Bacterial Stress Responses to Copper-based Coatings

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Healthcare-associated infections (HAIs) are a major concern in healthcare environments, where contaminated high-touch surfaces can act as persistent reservoirs for pathogen transmission. Among the leading causes of HAIs are the ESKAPEE pathogens which are notable for their survival on surfaces and high capacity to develop resistance to a wide range of antibiotics. To help limit the spread of HAIs, antimicrobial materials such as Copper (Cu) have been explored as alternatives to conventional surface materials. Advances in spray technology have enabled the development of cold-spray copper (CuCS) coatings which offers an emerging approach to create effective, durable, and low-cost coatings for surface fabrication. By depositing metallic powders below their melting temperature onto a ductile substrate, cold-spray technology allows for a rapid and resource efficient method for applications in hospitals settings. While CuCS surfaces are promising, the mechanisms of Cu surface-mediated bacterial killing, including the roles of Cu homeostasis and cell envelope interactions, are not fully understood. In this study, we evaluate whether CuCS coatings can serve as an alternative to bulk Cu surfaces and investigate the bacterial responses induced by surface-mediated Cu toxicity, using *Pseudomonas aeruginosa* as a model organism. In parallel, we assess the roles of copper homeostasis and cell envelope interactions using targeted mutants in these pathways. To assess the antibacterial activity of all Cu surfaces, we optimized a robust and reproducible time-kill assay that identified bacterial contact area as a critical determinant of killing kinetics, requiring strict control for reliable interpretation of results. To assess the performance of CuCS coatings, we assessed the influence of spray deposition temperatures on bacterial killing kinetics. Despite differences in coating density and microstructure, all spray deposition temperatures of CuCS coatings exhibited consistent antibacterial performance. This analysis was then extended to a panel of clinically relevant ESKAPEE pathogens, where CuCS and bulk Cu surfaces demonstrated similar antibacterial activity across both Gram-negative and Gram-positive species. While killing kinetics varied between species, the overall trends remained consistent across surface types, supporting CuCS as an effective alternative to bulk Cu. To investigate the mechanisms underlying Cu surface toxicity, we are currently performing RNA-sequencing to compare transcriptional stress responses in *P. aeruginosa* under sublethal exposure to bulk Cu, CuCS coatings, and soluble ionic $CuSO_4$. To complement this, we evaluated mutants deficient in Cu homeostasis, targeting cytoplasmic (*copA1*, a Cu efflux ATPase; and *cueR*, a Cu-responsive transcriptional regulator) and periplasmic (*copR/S* two-component system) regulatory pathways. Notably, the periplasmic Cu sensor D*copS* exhibited increased survival on Cu surfaces, where the response regulator D*copR* showed killing comparable to the wildtype. In contrast, the cytoplasmic Cu homeostasis mutants (D*copA1* and D*cueR*) showed similar killing kinetics to the wildtype, suggesting less involvement in Cu surface-mediated toxicity. In addition, we are currently evaluating mutants deficient in LPS O-antigen biosynthesis to assess the role of outer membrane structure and charge in bacterial-Cu interactions. Overall, these findings demonstrate that CuCS coatings provide an effective alternative to bulk Cu while also suggesting Cu surface toxicity involves a multifactorial mechanism incorporating both intracellular and contact-mediated interactions.

Gen abstract type Poster

Section Type AEM

217 - Engineering a Synthetic Bacterial Consortium for Enhanced Biofilm Formation and PET Surface Attachment

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Polyethylene terephthalate (PET) is one of the most widely used synthetic plastics, yet its high resistance to biological degradation makes it difficult to recycle sustainably. Although engineered PET hydrolases have shown improved catalytic performance, converting these advances into effective whole-cell PET degradation systems remains challenging. A major limitation is the weak interaction between microbial cells and the PET surface, which can restrict enzyme access to the polymer. In this study, we developed a synthetic bacterial consortium to improve PET surface attachment and biofilm formation. The system combined *Escherichia coli* Nissle 1917 engineered to express PETase with a biofilm-forming *Pseudomonas putida* strain evolved for growth on ethylene glycol. Co-culture experiments with PET films showed that both extracellular enzyme targeting and consortium composition strongly influenced microbial attachment, with the greatest and most surface colonization observed when PETase secretion was combined with the biofilm-forming partner. Quantitative analysis showed that the engineered consortium produced approximately 1.5-fold more biofilm than the control. We further evaluated whether mechanical roughening of PET could improve surface availability for microbial attachment. Surface roughness analysis confirmed increases in key roughness parameters, which were accompanied by increased biofilm formation. Depending on the degree of roughening, biofilm biomass increased by 10-24% relative to smooth PET films. These results identify surface accessibility as an important and often overlooked barrier in PET biodegradation and suggest that improving both the biological system and the physical substrate interface may be necessary for more effective biological PET recycling.

Keywords: Polyethylene terephthalate (PET), synthetic bacterial consortium, biofilm formation, PETase, surface roughness

Gen abstract type

Poster

Section Type

AEM

219 - Engineering probiotic yeast to produce double-stranded RNA for the development of an RNA interference-based biopesticide to control darkling beetle population in poultry houses.

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Darkling beetles, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), are globally distributed pests in poultry facilities that eat chicken feed, contaminate food products, and carry more than 60 avian diseases. During pupation, beetles burrow into housing insulation and wooden supports, causing significant structural damage and economic losses for farmers worldwide. Currently, chemical pesticides are the most common strategy for controlling beetle populations as they are cheap and readily available. However, pesticides are non-specific, can harm farmers and beneficial insects, and continuous reliance on chemical pesticides has led to the rapid development of resistance in many beetles.

RNA interference (RNAi) offers a target-specific alternative to traditional pesticides and has been used to control various agricultural pests. RNAi works by targeting the mRNA of proteins essential for beetles' survival using double-stranded RNAs (dsRNA). Once ingested, dsRNA is processed into small interfering RNA (siRNA) in the insect and is recruited into an RNA-induced silencing complex (RISC), which allows for target mRNA binding and its subsequent degradation, effectively preventing translation. The lack of essential proteins will eventually lead to the death of the insect.

This project aims to develop a probiotic yeast strain that produces dsRNAs targeting essential genes across the developmental stages of the darkling beetle, to be used as a feed-additive-based biopesticide. As the effectiveness of an RNAi-based biopesticide depends on the quality of the target genes, the project begins by conducting transcriptomic analysis across developmental stages of the darkling beetle to identify stably expressed genes that are suitable RNAi targets. Simultaneously, dsRNA production will be optimized through vector design and altering production conditions, allowing for the accumulation of dsRNA in probiotic yeast. Then, the effectiveness of dsRNA and the dsRNA-producing yeast will be tested through killing assays. Once mortality is confirmed, the dsRNA-producing yeast will also be tested for shelf stability and fed to poultry birds to test for side effects. Finally, multiple dsRNA combinations will be tested across the beetles' developmental stages to maximize population control.

The development of this dsRNA-based biopesticide led to a highly effective, target-specific approach for controlling the darkling beetle population in poultry farms while advancing foundational understanding of darkling beetle transcriptomics across developmental stages. This biopesticide will also help significantly alleviate the economic burden on poultry farmers by reducing structural damage caused by larvae, decreasing the transmission of various avian diseases, and helping farmers create a safe work environment with minimal use of chemical pesticides.

Gen abstract type Poster

Section Type AEM

220 - Diverse Cell Envelope Architectures Across Chloroflexota Revealed by Cryo-Electron Tomography

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The bacterial cell envelope is a multi-layered structure that provides a barrier against environmental stress and mediates interactions between the cell and its surroundings. Two major envelope architectures have been characterized: monoderm bacteria, which possess a single membrane and thick peptidoglycan (PG) layer, and diderm bacteria, which contain an inner membrane (IM) and an outer membrane (OM) separated by a thin PG layer. Though most characterized bacterial phyla typically share the same envelope type, recent studies have demonstrated that there are diderm members within phyla previously understood to be monoderm.

Chloroflexota is an ancient and phylogenetically diverse bacterial phylum with historically poorly defined cell envelope architecture. Using cryo-electron tomography (cryo-ET), which preserves cells in a near-native state and enables direct visualization of membrane bilayers and ultrastructure, we surveyed representative species across major Chloroflexota clades. These analyses reveal substantial diversity in cell envelope organization, including monoderm envelopes with and without PG, proteinaceous S-layers, and highly layered envelope types. Notably, several members exhibit a second bilayer surrounding thin PG and the IM, consistent with a diderm-like organization within a phylum traditionally classified as monoderm.

These observations highlight a gap in our understanding of Chloroflexota cell envelope biology. The composition and origin of these envelope features remain poorly defined, including the identity and structural organization of S-layer lattice proteins, their anchoring mechanisms, and the molecular components of putative OM-like structures. The absence of canonical OM features suggests that these structures may represent a novel OM.

We combined cryo-ET with biochemical and proteomics approaches to investigate these diverse envelope types, including S-layer architecture in monoderms and putative OM-like structures. Membrane enrichment and surface labeling strategies were used to identify proteins associated with outer envelope structures.

Together, this work defines the diversity of cell envelope architectures across Chloroflexota, expanding current models of bacterial envelope organization and providing insight into the evolution of diderm bacteria.

Gen abstract type

Poster

Section Type

AEM

221 - Probing the Genetic Diversity of Integrative Mobile Genetic Elements in Enterobacteriaceae isolated from pigs and poultry in Canada

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Antimicrobial resistance is a crisis that needs to be addressed at multiple fronts along the One Health continuum. Bacteria from agricultural settings are contributing to human and animal diseases and are increasingly resistant to antibiotics. However, knowledge gaps exist regarding their contribution to AMR in clinical settings. Bacteria exchange multidrug resistance genes via mobile genetic elements (MGEs), such as conjugative and mobilizable plasmids, integrative and conjugative elements (ICEs), and integrative and mobilizable elements (IMEs). IncC conjugative plasmids and ICEs of the SXT/R391 family are distantly related phylogenetically and functionally. Both play a considerable role in the expansion of antibiotic resistance, not only via their multidrug resistance (MDR) gene cargo, but also because they drive the mobilization of MDR-carrying IMEs. Mobile elements evolve rapidly and we need tools to assess their risk and anticipate their dissemination.

We have developed predictive tools to probe the diversity of genetic elements residing in three known integration sites of IMEs in *Gammaproteobacteria*, namely *dusA*, *trmE* and *yicC*. The approach was deployed on 3,652 genomes of isolates from swine and poultry samples collected in six Canadian regions over a decade. We recovered a single presumptive IME in *trmE,* and multiple IMEs and prophages integrated in *dusA* (*Escherichia*, *Morganella*, *Proteus*) and *yicC* (*Aeromonas*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*). The elements were annotated and searched for patterns of protein-coding genes consistent with intra- and intercellular mobility. AcaCD and SetCD-responsive promoter sequences that are the hallmark of gene expression elicited by IncC plasmids and SXT/R391 ICEs, respectively, were identified, as well as *attL* and *attR* integration sites. The cooccurrence of IMEs, prophages, SXT/R391 ICEs and plasmids was investigated. Some strains displayed insertions in both *dusA* and *yicC*, as well as multiple plasmid types including IncC. No presumptive SetCD-responsive promoters were identified in the strains containing SXT/R391 ICEs, except in these ICEs. Conversely, SetCD-responsive promoters, but not AcaCD-responsive promoters, were found in strains hosting IncC plasmids. The diverse genetic elements uncovered most likely influence their hosts' biology as they are reservoirs of genes coding for antimicrobial resistance, bacterial immune defense, genetic regulators, virulence factors, DNA/RNA processing, and other genes for housekeeping and metabolic functions.

Coexisting families of mobile genetic elements in bacteria are constantly evolving in tandem with the microbial communities and environments that host them. This study provides predictive data to capture the MGE ecosystems in isolates of key enteric pathogens isolated from major Canadian agricultural sectors. Assessing the diversity of IMEs and their relationship with prophages, plasmids and ICEs is crucial to understanding the evolutionary pathway by which multi-drug resistance IMEs emerge and how they domesticated conjugative elements to ensure their own propagation. Science-based evidence on emerging MGEs is pivotal to building predictive models and generating mitigation approaches that address emerging threats (zoonotic and/or resistant pathogens).

Gen abstract type

Poster

Section Type

AEM

222 - Characterization of bacterial communities in six floor drains from a commercial meat processing plant

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Floor drains are essential components of food processing environments. They facilitate the removal of waste fluids and help prevent water accumulation. However, their structural design, location, and tendency to accumulate organic matter often make them difficult to clean and disinfect properly. This makes them potential reservoirs for spoilage and pathogenic microorganisms that could contaminate the products and compromise their microbial quality and safety. This study investigated the diversity and dynamics of microbial communities in six floor drains of a federally inspected pig abattoir using physicochemical analyses, bacterial counts, and 16S rRNA gene sequencing. Their variability was examined across three processing sections (polishing (PL1, PL2); evisceration (EV1, EV2); cutting, (CU1, CU2)) and within individual drains (covering grid (CG); inner surface above the water level (IS); drain water (DW)). Drains were sampled over three consecutive weeks after end-of-week cleaning ("post-cleaning": PC) and again before pre-operational procedures at the beginning of the following week ("pre-operation": PO; ~ 48 h later). Surfaces were aseptically sampled using sponges pre-moistened with 10 ml phosphate-buffered saline (PBS). Drain water was aseptically collected in sterile containers using a peristaltic pump. Drain water physicochemical parameters did not vary significantly in the cold cutting section between PC and PO. In the polishing section, however, conductivity increased (both $P \leq 0.04$) and dissolved O_2 decreased (both $P \leq 0.004$) significantly in both drains (PL1 and PL2) during the same period. In PL1, bacterial loads were consistently higher in DW than on surfaces, with significantly higher levels at PO than in PL2 ($P \leq 0.01$). Alpha diversity was highest in polishing drains, intermediate in the evisceration section, and lowest in cutting drains suggesting that richness and evenness decreased along the processing line ($P \leq 0.03$ for all four indices: Chao1, Observed, Shannon and Simpson). Beta diversity is significantly different between the two drains within each section across all metrics (all $P \leq 0.01$). However, it did not vary significantly between PC and PO when all drains were analyzed together but differentiates the bacterial community during that period when drain-specific analysis was performed (all $P \leq 0.03$). *Lactococcus*, *Micrococcaceae*, and *Micrococcales* were associated with PC samples, whereas *Acinetobacter*, *Pseudomonas*, *Comamonas*, and *Aeromonas* were characteristic of PO samples ($P = 0.001$, LDA > 2.5). Beyond the site-specific variations observed across drains and processing sections, this study identified a stable core microbiota composed primarily of *Acinetobacter*, *Rothia*, and *Enhydrobacter*. These genera were frequently detected in most samples, regardless of temporal or spatial locations, suggesting that they form a resilient ecological backbone within drain environments. Spoilage-associated and biofilm-forming bacteria, notably *Pseudomonas*, *Aeromonas*, and *Acinetobacter,* were prevalent after weekend shutdown periods. These findings demonstrate that floor drains must be managed as critical microbial reservoirs, requiring sanitation strategies specifically adapted to their design, local conditions, and operational shutdown periods to effectively limit microbial persistence and contamination risks.

Gen abstract type

Poster

Section Type

AEM

223 - Mobile Genetic Elements as Drivers and Predictors of Antimicrobial Resistance Across the One Health Continuum in *Enterococcus faecium*

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Antimicrobial resistance (AMR) in enterococci represents a critical One Health challenge, driven by the dissemination of mobile genetic elements (MGEs) and compounded by difficulties in accurately predicting resistance phenotypes from genomic data. In this work, we integrated complementary experimental and computational approaches to investigate both the mechanistic basis of resistance gene mobility and the biological validity of machine-learning (ML)-based genotype-to-phenotype AMR predictions in a high-priority enterococcal pathogen.

We characterized the abundance, structure, and conjugation capacity of MGEs carrying clinically relevant resistance genes including *vanA*, *tet(M)*, and *erm(B)* across enterococci from clinical, environmental, and agricultural sources. Using replicon typing alongside reference-based and reference-free clustering, we identified diverse plasmid- and transposon-associated MGEs and assessed their transfer potential through agar-plate mating experiments with *Enterococcus faecium*, *Enterococcus faecalis*, and *Enterococcus hirae* recipients. Long-read sequencing confirmed successful inter- and intra-species conjugative transfer. A *vanA* gene cluster from *E. faecium* VRE0008 was embedded in a large RepA_N-type plasmid containing a Tn1546-like transposon and demonstrated broad host range transfer, predominantly via modular movement of a ~40 kb region surrounding the *vanA* locus. Similarly, *erm(B)* in *E. faecium* NS0794 was located on a transferable RepA_N-type plasmid, while *tet(M)* from *E. faecium* Ent0189 resided on a Rep_Trans-like plasmid with features of Tn916 integrative conjugative elements, exhibiting primarily intra-species transfer. These findings reveal extensive modularity and host plasticity of enterococcal MGEs, underscoring their role in AMR dissemination across ecological boundaries.

In parallel, we evaluated the biological relevance of ML-based AMR predictions by integrating transcriptomic profiling of selected *E. faecium* isolates representing vancomycin-, doxycycline-, and erythromycin-resistant phenotypes. Differential gene expression under antibiotic exposure revealed transcriptional patterns concordant with ML predictions and known resistance determinants, including *vanA*, *tet(M)*, and *tet(L)*. Notably, genes associated with MGE maintenance and dissemination, such as those encoding conjugative transfer proteins, DNA relaxase NicK, type IV secretion system components, and the anti-restriction protein AardA, were consistently upregulated. This supported the mechanistic plausibility of ML-selected genomic features. In contrast, erythromycin-resistant isolates carrying *erm(B)* did not exhibit transcriptional responses to antibiotic exposure, suggesting regulatory or mutational mechanisms not captured by gene presence-absence models.

Collectively, these integrated findings demonstrate that MGEs serve as both primary drivers of AMR spread and informative biological signals for predictive modeling, and that coupling genomic, functional, and transcriptomic data can substantially improve ML-based AMR predictions. This work strengthens the mechanistic foundation for predictive AMR analytics while providing actionable insight into the dynamic processes facilitating resistance dissemination across species and habitats.

Gen abstract type Poster

Section Type AEM

224 - Understanding the Microbial Ecology of Organic Greenhouse Irrigation System Biofilms for Implementing Bacteriophage Treatments

Courtney McKellar[1], Morgan Nolan[1], Cezar Khursigara[2], Youbin Zheng[1], Marc Habash[1]

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Biofouling through microbial biofilms is a major contributor to clogging in greenhouse irrigation systems, reducing production efficiency worldwide. Biofilms consist of microorganisms, primarily bacteria, embedded in a self-produced matrix of extracellular polymeric substances that enables them to adhere to surfaces and makes them difficult to remove. If left unmanaged, clogging can lead to crop losses, greenhouse infrastructure issues and risks to plant or consumer health. Current treatment strategies rely on harsh physical or chemical treatments, and organic greenhouse producers face stricter treatment options that mainly include high-dose hypochlorite, hydrogen peroxide, and peracetic acid. These treatments are often ineffective, with biofilm regrowth occurring within days to weeks, and they require large volumes of water and extended system downtime to flush and recirculate treatments, making them unsustainable for many greenhouse operations.

One promising biological treatment for managing biofilms in irrigation systems is the use of lytic bacteriophages to target and eliminate specific biofilm-forming bacteria. However, major knowledge gaps currently limit the broad adoption of this treatment across all greenhouses, particularly limited knowledge of the microbial composition and diversity of irrigation system biofilms and bulk water, which is needed to design effective bacteriophage treatments. To address this, we characterized the microbial ecology of an organic lettuce greenhouse facility with known biofilm issues in its on-site potable-water irrigation system using a combination of culturing- and sequencing-based approaches.

Two sampling events were conducted on February 25 and July 16, 2025, to capture both low- and high-biofouling periods. Biofilm and water samples were collected from biofouling-prone zones, including from the boom water recirculation loop areas and 100-micron mesh filters from within the irrigation booms. Bacteria were isolated using culture-dependent methods and identified through full-length 16S rRNA gene sequences obtained from whole-genome sequencing. The biofilm-forming capacity of each uniquely identified bacterial isolate was assessed individually using an in vitro high-throughput biofilm assay. Microbial community composition in whole biofilm and bulk water samples was further characterized using 16S rRNA gene amplicon sequencing and compared to the cultured bacterial isolate collection.

Across both sampling events, 239 bacterial isolates were collected from seven biofilm samples and two water samples, which were plated on five different media types. Sixty-eight unique taxa were identified, with all isolates identified to the genus level. Biofilm testing of the uniquely identified isolates showed that 37 isolates were moderate or strong biofilm formers and were found either exclusively in biofilm, in water samples, or in both. Seven genera, *Bacillus, Flavobacterium, Hydrogenophaga, Janthinobacterium, Pseudomonas, Xanthobacter,* and *Zoogloea*, were among the most abundant genera identified within the 16S rRNA amplicon sequencing data and were also identified within the isolate collection. This research is among the first to characterize the microbial composition of biofilms in potable water irrigation systems using both culture-dependent and culture-independent methods. This research will also inform the development of proof-of-concept bacteriophage treatments targeting three genera (*Pseudomonas*, *Janthinobacterium*, and *Zoogloea*) in subsequent studies.

Gen abstract type Poster

Section Type AEM

235 - DARTE-QM: A targeted metagenomic platform of antibiotic resistance genes for surveillance and risk assessment in a One Health context

Daniel Amoako[1], Gabhan Chalmers[1], Kerry Schutten[1], Brandon N. Lillie[1], Heather M. Murphy[1], Claire M. Jardine[1], Dan Tulpan[2], Brian P. Alcock[3, 4], Marcela Popa[5], Stefanie Glaeser[6], Dipen Pulami[6], Andrew Scott[7], Heike Schmidt[8, 9], Andrew G. McArthur[3, 4], Adina C. Howe[10], Nicole Ricker[1]

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Surveillance of antibiotic resistance genes (ARGs) across human, animal and environmental sectors can aid in our understanding of ARG transfer and persistence between geographical regions and within ecosystems. Current surveillance methods rely on culturing isolates, which can be labour-intensive and limited to a small number of clinically relevant pathogens. More recently, metagenomic sequencing methods are being employed to determine the total resistome of diverse environmental systems, which has the benefit of capturing resistance information from commensal bacteria as well as pathogens. However, metagenomic surveillance efforts are limited by the high cost of obtaining sufficient sequencing depth to fully capture ARGs, and there are no established criteria for developing actionable risk metrics for AMR from resistome data.

To facilitate broad ARG surveillance at a reduced cost per sample, we have developed a targeted amplicon-based ARG platform (DARTE-QM) for use on environmental samples. Recognizing that environmental surveillance needs differ from clinical data needs, we designed the platform to target 379 genes considered high-risk of potential mobility into pathogens, capable of capturing over 3393 gene variants based on the CARD database. Compared to total resistome sequencing, DARTE-QM data is targeted towards antibiotic inactivation mechanisms and against intrinsic genes such as membrane permeability and efflux pumps. Gene targets were prioritized according to known associations with mobile genetic elements and/or prevalence data from the CARD database supporting presence in a diversity of bacterial taxa. Samples were normalized by qPCR to 16S rRNA gene abundance prior to sequencing and an internal spike-in control has been incorporated for normalization between sample types and across runs. The platform utilizes Illumina AmpliSeq technology and short-read sequencing to amplify a 300 bp fragment of the target genes. Within the highly diverse beta-lactamases, some gene targets can only be identified to the family level whereas other groups can be distinguished to the individual genes based on the amplicon sequence variation.

We have tested the platform on diverse samples including soil, groundwater, manure, wastewater, fecal samples from chickens and fecal swabs from gulls. Robust results are obtained when DNA is not limiting, but AMR genes could still be detected when 16S rRNA values were below qPCR detection limits. Samples show distinct resistance gene patterns according to source and reproducible diversity results between samples across runs. Dairy manure samples examined from 4 countries demonstrated consistency in gene detections and were supported by cultured isolate data and shotgun metagenomics obtained from the same sources. Extended spectrum cephalosporin genes were detected at low levels from these dairy samples, consistent with the use of antibiotic selection to enrich for these isolates in the original samples. Wastewater samples were found to carry substantially higher abundance of these genes, and greater overall beta-lactamase diversity compared to animal sources. Current work is underway to correlate DARTE-QM abundance data with culturable detection limits and the next steps will be to develop risk assessment models based on ARG detection.

Gen abstract type

Poster

Section Type

AEM

238 - Beyond clinical surveillance: wastewater-based detection of sustained respiratory virus activity in unhoused populations

Daniel Crowley[1], Nicole Acosta[1], Barbara Waddell[1], Casey Hubert[1], Michael Parkins[1], Maria Chavarriaga[1], Paul Westlund[2], Summie Lo[1], Chloe Papparis[1], Alex Buchner Beaudet[1], Danielle Southern[1], Tyler Williamson[1], Rhonda Gail Clark[1], Xiaotian Dai[1], Xuewen Lu[1], Jia Hu[3], Benson Weyant[4], Jason Cabaj[4], Jamil Kanji[4], Kevin Frankowski[1], Christine O'Grady[1], Bonita Lee[5], Judy Qiu[6], Lilly Pang[5], John Conly[4]

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Introduction: Respiratory viruses are major drivers of healthcare utilization and system strain, necessitating robust surveillance to characterize their epidemiology and inform public health. However, conventional case-based surveillance, anchored in clinical testing, primarily captures those who seek care, thereby underrepresenting mild, asymptomatic, and structurally marginalized populations. Unhoused populations experience disproportionate morbidity and mortality from respiratory viruses, driven by crowded shelter conditions, limited ventilation, comorbidity burden, and barriers to healthcare access. Wastewater-based surveillance (WBS) provides a population-level approach that captures pathogen shedding independent of testing access. Shelters represent ideal near-to-source sampling environments, characterized by concentrated populations, discrete catchments, yet remain largely absent from the WBS literature. Here, we evaluate WBS of respiratory viruses in urban shelters relative to municipal wastewater and clinical surveillance.

Methods: Composite 24-hour wastewater was collected one to three times weekly from two urban shelters (Shelter-1: ~900 beds/night; Shelter-2: ~100 clients/day, substance use focus) and one municipal wastewater treatment plant (WWTP; serving ~1.04 million residents) in Calgary, Alberta, from July 2022 to October 2025. Influenza A virus (IAV) and respiratory syncytial virus (RSV) RNA were quantified by RT-qPCR and normalized using Pepper Mild Mottle Virus (PMMoV) as a fecal-strength covariate. Viral dynamics were modelled using Bayesian hierarchical state-space models with cubic B-spline latent trends and a hurdle likelihood for non-detects; location-specific and pooled models were compared via leave-one-out cross-validation (ELPD). Wastewater trends were used to regress weekly clinical case counts, comparing models with and without shelter-level signals. At Shelter-1, temperature and overnight bed occupancy were included as covariates.

Results: Of 621 samples, 90% yielded detectable IAV RNA and 67% detectable RSV RNA. Location-specific models outperformed pooled models for both pathogens (IAV: ΔELPD = −141.7, SE = 15.4; RSV: ΔELPD = −44.6, SE = 14.5), confirming that shelters and the municipal WWTP have epidemiologically distinct signals. IAV shedding was 2.2-fold higher during respiratory virus season than off-season at shelters, compared to 3.9-fold at the WWTP; discrete off-season outbreaks were detected at shelters in summer 2023 and spring 2025. RSV exhibited more pronounced and comparable seasonality across both shelters and the WWTP. Municipal wastewater positively predicted city-wide IAV and RSV case counts. Adding shelter-level wastewater produced only marginal improvements in case model fit; notably, the shelter signal was *negatively* associated with city-wide cases after adjusting for the municipal signal, reflecting off-season shelter activity when reported cases were lowest. After adjusting for temperature, overnight occupancy showed no independent association with viral concentrations (IAV: β= −0.13, 95% CrI [−1.92, 1.71]; RSV: β= 0.37, 95% CrI [−1.45, 2.19]). Temperature was negatively associated with RSV (β= −0.05, 95% CrI [−0.09, −0.01]).

Conclusions: Near-to-source WBS reveals year-round respiratory virus dynamics in shelter populations missed by clinical surveillance and municipal wastewater monitoring. The inverse association between shelter- and city-level signals supports a surveillance gap, whereby transmission among unhoused populations goes undetected by conventional surveillance. Building-level WBS offers a low-barrier, passive tool for monitoring this burden and evaluating infection prevention and control interventions (IPC). Sustained investment in year-round IPC, vaccination, and shelter-based surveillance infrastructure is warranted.

Gen abstract type Poster

Section Type AEM

240 - Multi-omic Investigation of Microbial Community Dynamics in Mixed Fermentation Sour Beer

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Spontaneous fermentation in sour beer relies on a diverse microbial community that produces complex, sour flavours, contributing to its premium value. However, spontaneous fermentation has several challenges, including poor process control, inconsistent product quality, long fermentation times, and product waste from failed fermentations. This study explored the use of mixed controlled fermentation in the production of sour beer. While controlled fermentation has been extensively studied in fermented beverages such as wine, its use in sour beer production remains poorly understood. Although the core microbial community in spontaneous beer is well characterized, the dynamics of microbial assembly, gene expression, and metabolite changes throughout controlled fermentation remain uncharacterized. To comprehensively understand controlled fermentation, this study combined RNA sequencing and volatile metabolite profiling via GC×GC-TOFMS across 10 barrels from two batches, sampled at six time points across one year of fermentation (1 week, 2 weeks, 3 months, 6 months, 9 months, and 12 months). The transcriptomic dataset showed that community assembly was similar across batches and barrels. However, community structure shifted over time: the community at one and two weeks had little to no *Brettanomyces* gene expression, whereas later time points were dominated by this organism. Overall microbial diversity was lower in mixed fermentation than in spontaneous fermentation, particularly during the early stages, when no enterobacteria were observed. Apart from the lack of enterobacteria during the early phases, community assembly resembled spontaneous beer with an initially high level of *Saccharomyces* and lactic acid bacteria, followed by *Brettanomyces* dominating the community. Differential expression analysis identified 24,223 genes with significant changes in expression throughout fermentation. The highest gene activity was observed during early fermentation, particularly in the first two weeks. Key metabolic pathways, including ethanol production, maltose assimilation, beta-glucosidase release and ester production, were upregulated at these early stages. These findings indicate that controlled mixed fermentation shares many similarities with spontaneous fermentation; however, there are distinct differences during the early phases. The high level of gene activity early in the process also indicates that fermentation may proceed more rapidly. To improve taxonomic classification accuracy, we are evaluating multiple pipelines. In parallel, metabolomics data are being analyzed to examine changes in metabolite profiles across fermentation stages. Overall, this study enhances our understanding of controlled mixed fermentation and demonstrates that it is a promising approach to sour beer production.

Gen abstract type

Poster

Section Type

AEM

241 - Study of the regulation of the type VI secretion system (T6SS) in *Cronobacter* spp

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The type VI secretion system (T6SS) is a contact-dependent molecular nanomachine that injects toxic effectors directly into neighbouring cells. In *Cronobacter* sp., the T6SS appears to be particularly active. Contrary to most T6SS, *Cronobacter*'s T6SS is active in rich media such as LB and does not require genetic manipulations or complex environments to be activated.

Here, we characterized the T6SS activity of *Cronobacter* sp. under various growth conditions. We observed T6SS killing activity in all media tested, including rich media (LB, BHI), minimal media with varied sources of carbon, as well as gut-mimicking media. Our results thus suggest a constitutive activation of the T6SS.

In addition, while most of our *Cronobacter* strains displayed constitutive activity, we also identified a few strains in our panel that did not show any T6SS activity. Genomic alignments made by software such as RStudio, Clinker, and Galaxy showed a highly conserved T6SS cluster with a few differences. We are currently exploring these differences. A better understanding of the regulation of *Cronoacter* T6SS will enable us to explore the use of a controlled T6SS antibacterial system.

Gen abstract type

Poster

Section Type

AEM

242 - Enhancing electron transfer in *Rhodopseudomonas palustris* 42OL photobio-cathodes through the redox mediator anthraquinone-2,6-disulfonate (AQDS)

Chiara Capelli[1], Karima Guehaz[1], Matteo Daghio[1], Matilde Ciani[1], Cecilia Petitta[2, 3], Carolina Cruz Viggi[2], Matteo Tucci[2], Federico Aulenta[2], Alessandra Adessi[1], Carlo Viti[1]

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The global energy landscape is currently facing unprecedented challenges. Escalating costs are putting significant pressure on the economic viability of the agri-food industries. At the same time, food processing residues with high Chemical Oxygen Demand (COD) pose a dual challenge as environmental pollutants and potential sources for resource recovery. Bio-electrochemical systems (BES) offer a promising solution in this context, having emerged as a transformative technology for converting organic waste into valuable bioproducts and sustainable energy carriers. Among electroactive microorganisms, purple non-sulfur bacteria, such as *Rhodopseudomonas palustris* 42OL, are notable for their exceptional metabolic versatility. When integrated into a photobio-cathode, these bacteria can theoretically utilize cathode-derived electrons to drive photo-H_2 production and biomass synthesis, potentially coupled with anodic oxidation of waste streams. However, the efficiency of these systems is often limited by extracellular electron transfer (EET) at the microbe-electrode interface.

This study investigates the use of anthraquinone-2,6-disulfonate (AQDS) as a redox mediator that mimics natural humic substances. The aim is to bridge the gap between a polarised cathode and *R. palustris* 42OL, establishing whether mediated EET could significantly increase H_2 yields and biomass growth in a BES. *R. palustris* 42OL was inoculated into the cathodic chamber, and a constant potential of -600 mV *vs*. Ag/AgCl was applied for 384 hours to provide a stable reducing environment. Five conditions were compared in quadruplicate: (i) mediated BES; (ii) mediator-free BES; (iii) photo-fermentation with AQDS; (iv) photo-fermentation without AQDS; and (v) abiotic controls. Growth kinetics were analyzed using the Baranyi and Roberts model, while acetic acid consumption and gas production were quantified via HPLC and GC-FID, respectively.

The results revealed a distinct interaction between the mediator and *R. palustris* 42OL. Notably, the highest biomass density was observed in the mediator-free BES (OD_{660} = 2.56 ± 0.01), which exhibited the shortest lag phase and the most efficient acetic acid depletion (residual concentration of 0.50 ± 0.24 g/L). However, the presence of AQDS was crucial for H_2 production, achieving a cumulative production of 141.93 ± 7.71 mL. Furthermore, H_2 yield improved in the mediated BES, reaching 0.15 ± 0.01 mmol H_2 mol^{-1} of acetic acid. From an electrochemical perspective, the total cumulative charge reached 142.70 ± 40.20 C, representing a 17-fold increase in electron recovery compared to the mediator-free control. Cyclic voltammetry suggested that the quinone lowered the kinetic barriers for electron uptake, thereby improving the microbe-electrode interaction. This research demonstrates that, although AQDS redirects metabolic flux away from biomass accumulation, it acts as a powerful catalyst for bio-H_2 synthesis in photobio-cathodes. By facilitating efficient electron shuttling, AQDS bridges the gap between inorganic electrodes and *R. palustris* 42OL. This approach offers a robust strategy for optimizing BES performance and aligns biotechnological innovation with the goals of a circular bioeconomy by converting food industry waste into clean energy.

This work was supported by Ministero italiano dell'Università e della Ricerca during the research program PRIN 2022 PNRR "Valorization of cheese whey by hydrogen production in bio-electrochemical systems catalyzed with purple bacteria (WHISPERNo. P2022W4MNM)".

Gen abstract type Poster **Section Type** AEM

246 - Microbial diversity and transport in Arctic wildfire smoke plumes

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Climate change and other anthropogenic factors are impacting fire regimes and increasing the risk of wildfires globally. Areas experiencing outsized effects of climate change such as the Arctic are predicted to experience significant increases in wildfire activity as temperatures increase and biomass becomes drier. Concomitant with wildfire will be an increase in area of smoke-affected regions and higher frequency of smoke events affecting large and distant geographic areas. Wildfire smoke plumes can contain elevated concentrations of airborne microorganisms aerosolized during burning, many of which may remain viable and can be transported long distances. We sampled air during a wildfire event in the Northwest Territories of the Canadian Arctic in 2023. We sampled in two locations affected by wildfire smoke (maximum PM2.5=427 ug/m3, PM10=771 ug/m3) located ~12 and ~88 kms from the wildfire by filtration onto an electrostatic filter. DNA was extracted from the filters and 16S rRNA gene and ITS amplicons were sequenced. Soils and the phyllosphere of dominant plant matter from the sampled areas were also sequenced to distinguish smoke plume microorganisms from locally aerosolized microorganisms. Smoke-influenced airborne microbiota were distinct from local source microbial communities, as well as non-smoke-influenced airborne microbiota, and were enriched in fungal and bacterial species identified in the burn site phyllosphere. Microorganisms aerosolized in smoke were dominated by plant pathogens and enriched in human fungal pathogens, indicating potential harmful impacts on downwind environmental and built ecosystems. Improved understanding of microorganisms in wildfire smoke will help predict effects of increasing wildfire activity on downwind environments.

Gen abstract type

Poster

Section Type

AEM

249 - Feeding the beast: Secondary bacterial invaders drive growth of *Melissococcus plutonius in vitro*

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European foulbrood stands out as one of the most pervasive and economically impactful honey bee diseases, affecting colonies in virtually every region where apiculture is practiced. The etiological agent, *Melissococcus plutonius*, is a Gram-positive bacterium that proliferates within the midgut of infected larvae. Infection disrupts development and often results in mortality. Although *M. plutonius* has been identified as the primary pathogen, symptomatic larvae are frequently co-infected with bacteria such as *Paenibacillus* spp, referred to here as 'secondary invaders'. Despite their frequent co-isolation, the mechanisms driving these polymicrobial interactions remain poorly understood.

This study investigated whether secondary invaders secrete metabolites that enhance *M. plutonius* growth through metabolic cross-feeding. We cultured *M. plutonius* strain LCAL0054 in media supplemented with cell-free supernatant from *Paenibacillus alvei* (DSM 29 and CA0065) and the recently classified *Paenibacillus melissococcoides* (CA0005). Supplementation with cell-free supernatant at concentrations of 12.5-50% (v/v) from all three strains significantly enhanced *M. plutonius* growth. Treated cultures reached optical density (OD_{600}) values exceeding 1.4, compared to minimal growth (0.1-0.4) in unsupplemented controls. Colony-forming unit (CFU) counts validated these findings, demonstrating a 2-log increase in viable cells, peaking at approximately 1×10^8 CFU/mL.

To identify specific metabolites responsible for growth enhancement, we analyzed *Paenibacillus* supernatants using proton nuclear magnetic resonance (1D ^{1}H-NMR) spectroscopy. We identified three predominant metabolites produced by all strains: formate (21-22 mM), acetate (15-18 mM), and ethanol (6-11 mM). Targeted metabolite supplementation could not recapitulate the whole supernatant effect on *M. plutonius* growth and revealed paradoxical results. Compared to unsupplemented controls, acetate and ethanol slightly inhibited *M. plutonius* growth, while formate enhanced growth only at supraphysiological (2×) concentrations. However, growth with formate supplementation remained significantly lower than that observed with whole supernatants (OD_{600}~0.76).

This study provides evidence that cell-free supernatants from secondary invaders directly enhance *M. plutonius* growth in vitro. However, acetate, formate, and ethanol are unlikely to be the primary mediators of the observed cross-feeding interactions. Efforts to characterize the mechanism underlying the observed synergy are currently ongoing. One possible explanation is that secondary invaders may supply essential precursors for which *M. plutonius* is auxotrophic. Based on evidence of genome reduction in *M. plutonius*, this interaction may involve low-abundance growth factors such as amino acids, vitamins, or signaling molecules, that are not readily detectable and require more sensitive detection methods for identification.

These findings may have implications for European foulbrood management strategies. Current approaches targeting only the primary pathogen may be insufficient, as secondary invaders could create metabolic niches that lower the threshold for clinical symptoms to emerge. Future therapeutic interventions could target the broader microbial network that supports pathogen growth. For example, through selective antimicrobial strategies or supplementation with competing organisms to disrupt metabolic activities of secondary invaders that are critical for pathogen persistence.

Gen abstract type Poster **Section Type** AEM

251 - Investigating Interkingdom Interactions in Kefir

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Kefir is a fermented milk beverage that has been produced and consumed for thousands of years. Traditionally, kefir is fermented using kefir grains, which are complex matrices of polysaccharides and proteins that are colonized by a variety of yeast and bacterial species. The interactions that occur between the yeast and bacterial species in kefir grains creates a complex product associated with an array of potential health benefits, such as the ability to lower plasma cholesterol. Previous work has established that the health benefits of traditional kefir can be recapitulated by a model community of bacteria and yeast commonly found in kefir fermentations. Additionally, it has been established that the microbial composition of kefir plays a critical role in the associated health benefits. In particular, this work established that both the lactobacilli and yeast populations are essential for the observed health benefits. While previous work has established a range of interactions between the bacterial species present in kefir, the potential for interkingdom interactions in kefir has not yet been investigated.

For this reason, we investigated specific interactions between 2 lactobacilli species (*Lactobacillus kefiranofaciens* and *Lentilactobacillus kefiri*) and 4 yeast species (*Saccharomyces cerevisiae, Pichia fermentans, Kazachstania unispora,* and *Kluyveromyces marxianus*) present in the previously established model kefir community. Pairwise interactions were measured in both liquid and solid media to determine how these microbes interact in both the spatially constrained condition of the kefir grain and the planktonic conditions in milk.

To characterize how these microbes interact, growth performance, acidification rate, and colony morphology assays were performed. Our preliminary results indicate that there are interactions between both lactobacilli species and the 4 yeast species tested. Particularly, our results indicate there is likely a strong positive interaction between *L. kefiri* and *K. marxianus* in both liquid and solid media, resulting in increased *L. kefiri* growth rates in both conditions. Furthermore, we found that *L. kefiri* is unable to grow on solid media unless it is co-cultured with one of the 4 yeast species. We also observed that in liquid media, the growth rates of the 4 yeast species were never strongly increased by co-culturing with either of the 2 lactobacilli species. Despite this, in general, we observed stronger positive interactions between the yeast and lactobacilli species in liquid media as opposed to solid media. Suggesting that more interspecies cooperation occurs in milk as opposed to in the kefir grain. To further characterize the intraspecies interactions identified, we will examine metabolic production using high-performance liquid chromatography to determine differences in carbohydrate and organic acid metabolism.

The results from this project improve our understanding of interkingdom interactions in kefir and food fermentations in general, while providing insight into the specific interactions that are essential for maintaining the health benefits associated with this product. Establishing positive and negative interactions in this community lays the groundwork for future research to delve into specific metabolic pathways involved and answer mechanistic questions surrounding these interactions.

Gen abstract type

Poster

Section Type

AEM

253 - Synergistic Shutdown and Evolutionary Restriction: Proteomic and Genomic Insights into Phage Cocktail Efficacy Against Shiga Toxin-Producing *Escherichia coli*

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Background: The transmission of Shiga toxin-producing *Escherichia coli* (STEC), particularly serotype O157:H7, from beef cattle to humans represents a critical One Health challenge. These infections frequently lead to severe conditions such as hemolytic uremic syndrome (HUS). This persistent threat necessitates novel, effective on-farm interventions to ensure food safety. While virulent bacteriophages offer a promising alternative to traditional antimicrobials, their therapeutic potential is hindered by the rapid evolution of bacterial resistance and a lack of understanding regarding the complex phage-bacterium interplay. Furthermore, the mechanisms of multi-phage synergy—specifically how cocktails coordinate host alterations to limit bacterial escape—remain a critical knowledge gap.

Objectives: Building upon previous *in vitro* findings demonstrating that a defined four-phage cocktail (T1, T4, T5, and rV5 types) inhibits STEC O157 more effectively than its individual components, this study aimed to define the underlying mechanisms of this synergy. We aimed to elucidate the combined molecular, metabolic, and genetic strategies the cocktail employs to hijack cellular machinery, suppress anti-phage mutant development, and impose growth fitness costs.

Methods & Results: We utilized a comprehensive dual-omics approach: proteomics to evaluate time-dependent metabolic shutdown, and whole-genome sequencing (WGS) to assess mutational landscapes, defense systems (DSs), and fitness costs following a five-day repeated phage exposure time-kill assay. Proteomic data revealed massive host remodeling. While single phages (T5, rV5) suppressed host ribosome activity by 30 minutes, the four-phage cocktail was vastly more effective at 60 minutes in dismantling core host regulatory networks (DNA, RNA, ATP, iron binding) and reducing anaerobic respiration proteins. Additionally, proteomics identified six highly abundant phage proteins driving late-stage infection, including known structural/membrane proteins alongside four uncharacterized targets.

Concurrently, the cocktail demonstrated superior evolutionary restriction. Of 11 phage-resistant O157 isolates analyzed, ten emerged from single-phage treatments, one from a three-phage cocktail, and zero from the four-phage cocktail. Most mutants (10 of 11) exhibited reduced growth fitness compared to the ancestral strain. WGS revealed that single-phage mutants underwent extensive genomic changes (SNPs, indels, and gene absences), frequently affecting core genes associated with fatty acid metabolism (*fadH*), stress response (*YgiW*), and the HokC toxin-antitoxin system. Conversely, the single mutant from the three-phage cocktail exhibited no gene loss and contained SNPs in only two genes associated with membrane proteins and lipopolysaccharide biosynthesis. Furthermore, while genomes consistently harbored between 19 and 22 antiviral DSs—including 5 to 7 CRISPR-Cas systems—none possessed spacers matching the phage genomes, and the correlation between DS counts and phage infectivity remained weak.

Conclusions: High-efficacy phage cocktails address the global challenge of STEC by operating through a potent dual mechanism. They synergistically shut down host metabolic machinery more efficiently than individual components while simultaneously minimizing the genetic mutations and gene loss events that drive bacterial resistance. These findings emphasize that the ability to profoundly suppress host transcription, dismantle resistance networks, and severely limit evolutionary escape pathways should serve as primary criteria for selecting agricultural biocontrol and therapeutic candidates.

Gen abstract type

Poster

Section Type

AEM

254 - Syntrophic partnerships between methanotrophs and non-methanotrophic methylotrophs for increased methane oxidation

Rachel Weitz[1], Dominic Sauvageau[1], Lisa Stein[1]

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Methane is a potent greenhouse gas whose relatively short atmospheric lifetime makes it an actionable target for mitigating near-term climate warming. Mitigation of methane could be partly accomplished by bacteria that consume methane, known as methanotrophs. These bacteria comprise the biological methane sink and have been studied for potential applications in climate change mitigation strategies. However, their applications are currently limited due to their slow rates of methane oxidation at atmospheric methane concentrations.

In their natural environment, methanotrophs grow in partnerships with many other microorganisms. A group of microbes that have been observed to grow alongside methanotrophs are the non-methanotrophic methylotrophs, which are capable of utilizing one-carbon compounds other than methane. Despite their frequent co-occurrence, methanotrophs have primarily been studied in pure cultures, overlooking the contribution of interactions with other organisms, such as methylotrophs, to their growth, gene regulation, and methane oxidation capabilities.

This research investigates how methanotrophs behave in co-cultures with methylotrophs and assesses the effect of this partnership on methane oxidation. Central to this work is the establishment of co-cultures of *Methylomicrobium album* BG8 (a methanotroph) and a *Methylobacterium* sp. (a pink-pigmented facultative methylotroph), which have been previously observed to grow together. The co-cultures are fed methane as the sole carbon and energy source, requiring the methylotroph to rely on metabolic products of the methanotroph. Co-cultures were established at varying inoculation ratios and methane and oxygen concentrations. Higher inoculation ratios of methylotroph to methanotroph showed more success in establishing a co-culture, as indicated by observable growth of pink-pigmented cells. Quantitative polymerase chain reaction (qPCR) assays that differentiate between the two species were developed to enumerate cells. The methanotroph-specific *pmoA* gene encoding the particulate methane monooxygenase was used to enumerate *M. album* BG8, while the phosphoenolpyruvate carboxylase gene was used to enumerate the *Methylobacterium* sp.

Ongoing work focuses on using the qPCR assays to track population dynamics in the co-cultures, measuring methane oxidation rates to evaluate whether co-cultures utilize methane more efficiently, and using a multi-omics approach to investigate the cross-feeding relationship between the species. Overall, this research broadens understanding of methanotrophs in natural partnerships with methylotrophs and evaluates the potential of methanotroph-methylotroph co-cultures to increase methane oxidation. These findings may help inform the development of microbial strategies for improving methane removal and mitigating near-term climate warming.

Gen abstract type

Poster

Section Type

AEM

257 - Dietary malate supplementation induces dose- and time-dependent shifts in the rumen microbiome of pregnant cows

Devin Holman[1], Katherine Gzyl[1], Hushton Block[1]

[1]Agriculture and Agri-Food Canada

Malate is a naturally occurring organic acid and approved feed ingredient that has been proposed as an alternative strategy for modifying rumen fermentation and microbial ecology. As an intermediate in the succinate-propionate pathway, malate can stimulate lactate uptake by *Selenomonas ruminantium*, potentially reducing acidosis risk. It can also be converted to fumarate, an electron acceptor that may reduce hydrogen availability for methanogenesis while favouring propionate production. Although previous studies have reported inconsistent effects of malate or fumarate on methane mitigation, responses appear to depend on dose, fermentation conditions, and microbial interactions. We therefore evaluated whether dietary malate supplementation alters rumen microbial composition and functional potential in vivo. Rumen samples were collected from cows assigned to one of three diets: control (no malate), 1.5% malate, or 3% malate, at baseline (day 0) and on days 28 and 70.

Malate supplementation altered rumen microbiome composition in a time-dependent manner, with the strongest differences observed at day 70 between the control diet and both malate-supplemented groups (3% malate: R2 = 0.10; 1.5% malate: R2 = 0.12; $P < 0.05$). The 3% and 1.5% malate groups also differed at day 70 (R2 = 0.10; $P < 0.05$). Differences at day 28 were more modest ($R^2 = 0.08$ to 0.10) and did not reach significance ($P > 0.05$). These community-level shifts were driven by changes in the relative abundance of multiple microbial species, including enrichment of *Slackia heliotrinireducens*, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, and *Megasphaera elsdenii* in the 3% malate group at day 70. *Prevotella ruminicola* was among the species enriched in the 1.5% malate group relative to the control group, while *Succiniclasticum ruminis* was reduced. Methanogen-associated responses were mixed, with reduced abundance of *Methanobrevibacter ruminantium* but increased *Methanoprimaticola* sp015063285 in the 3% malate group at day 28, and increased *Methanocatella* spp. at day 70. Despite these taxonomic shifts, no carbohydrate-active enzyme (CAZyme) families were differentially abundant among diets at any sampling day, suggesting that functional redundancy may have masked treatment-related differences in broad CAZyme profiles. Overall, these results show that malate supplementation had dose-dependent and time-dependent effects on the rumen microbiome, with the greatest effects observed at the end of the trial. Species-level changes were evident even though CAZyme family profiles remained stable, indicating that dietary effects on the rumen microbiome may be easier to detect at the taxonomic level than at the broader functional level.

Gen abstract type

Poster

Section Type

AEM

259 - Two Oxygen-Responsive N_2O Production Pathways Drive Ecological Niche Differentiation in Ammonia-Oxidizing Bacteria

Helin Wang[1,2], Sujani Gomes[1], Aderonke Adigun[1], Ping Li[2], Lisa Stein[1]

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Nitrous oxide (N_2O) is a potent greenhouse gas accounting for ~7% of global radiative forcing, with ammonia-oxidizing bacteria (AOB) as key biological sources. Yet how oxygen availability governs their metabolic strategies and ecological specialization remains poorly understood. Using *Nitrosomonas europaea* ATCC 19718 and *Nitrosomonas communis* NM2 as representative models, this study reveals two mechanistically distinct, oxygen-responsive N_2O production patterns through an integrated multi-omic approach.

Comparative transcriptomic analysis identified 1,923 transcripts in *N. europaea* and 1,734 in *N. communis*, while proteomics detected 1,098 and 1,175 proteins, respectively. Under micro-oxic conditions, *N. europaea* activates a canonical nitrifier-denitrification pathway mediated by NsrR, NorB, and NirK (Type I), in which intracellular NO accumulation and ROS stress drive reductive N_2O formation. In contrast, *N. communis* operates via an oxic, hydroxylamine-dependent route (Type II) centered on the CytL-CccB-McoP module. Despite harboring a *norCBQD* gene cluster, *N. communis* expressed no NOR proteins under either condition, instead relying on cytochrome P460 (CytL) and cytochrome c551 (CccB) for electron transfer, with strong co-expression between *cytL* and *cccB* (Spearman's $\rho = 0.88$). Heterologous expression of the multicopper oxidase *mcoP* in *E. coli* confirmed its functional role in N_2O turnover, achieving approximately 68% higher N_2O reduction efficiency compared to controls.

To assess the ecological relevance of these two patterns, we surveyed 1,013 environmental metagenomes across five biome types (ocean, freshwater, soil, wastewater, and marine sediment) using the MGnify database. The Type II marker *cytL* showed highest prevalence in freshwater (99%) and soil (97%) environments, consistent with aerobic habitats. In contrast, the Type I marker *norB* was most prevalent in wastewater (99%) and ocean (48%) systems, but was rarely detected in soil (20%) or freshwater (5%), consistent with its role in micro-oxic nitrifier denitrification. This striking biome-specific partitioning mirrors the physiological divergence observed between the two strains in pure culture, confirming that oxygen gradients act as a primary ecological driver shaping AOB community composition and N_2O flux potential. Pan-genomic analysis of 311 cultured bacterial genomes spanning 11 phyla further revealed that both pathway markers extend well beyond canonical AOB lineages, with 225 genomes harboring *cytL* and 100 harboring *norB*, suggesting cross-phylum dissemination via horizontal gene transfer.

Together, these findings sketch an integrative oxygen-responsive framework connecting molecular regulation to ecological adaptation in AOB. Type I AOB function as electron sinks under fluctuating, low-oxygen conditions, while Type II AOB maintain redox stability through oxygen-dependent electron relay systems in well-aerated habitats. This mechanistic duality provides a new basis for predicting AOB-driven N_2O dynamics across diverse ecosystems under changing environmental oxygen conditions.

Gen abstract type Poster **Section Type** AEM

260 - When Laundry Pods Meet Poop: Wastewater Microbe Responses to Polyvinyl Alcohol Plastics

Mileena Sabarinath[1, 2], Jonas Stadfeld[1], Abdoullah Hleihel[1], Srijak Bhatnagar[1, 2]

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Polyvinyl alcohol (PVA) is a water-soluble plastic polymer widely used for detergent pods, paper products, construction materials, and biomedical products. With increasing adoption of detergent pods, such as laundry pods and dishwasher pods, due to convenience, there has been an increase in PVA discharged into municipal systems. Because of its solubility, PVA is expected to be more readily biodegraded, but the wastewater microbes that can carry out degradation and the effects of PVA on wastewater microbial communities remain poorly understood. Hence, we present a study investigating the effect of PVA on wastewater microbial community composition and resulting metabolic activity.

For this, replicate aerobic microcosms containing Bold's Basal Media (carbon-limited) and 2% w/v commercially used laundry pods PVA were inoculated with 1% v/v secondary effluent from a wastewater treatment plant in a major city in Canada at 20°C. Simultaneously, control microcosms containing no PVA or no inoculum were also created. At 2, 7, and 9 days post-inoculation, CO_2 and O_2 of the microcosms were measured using gas chromatography, before sacrificial sampling for cell counting, microbial community composition, and GC-MS. From filtered media, DNA was extracted using the Qiagen PowerLyzer PowerSoil Kit and sequenced using the Nanopore MinION for near full-length 16S rRNA gene sequencing to assess community composition and metagenome for metabolic reconstruction.

We found that while alpha diversity (Shannon index) did not differ significantly between treatment and controls, the CO_2 and cell counts indicated microbial growth on PVA compared to controls. Moreover, the XX ordination, using YY distance measure, showed that the treatment community was significantly different from the control and the wastewater inoculum. We observed both qualitative and quantitative decreases in PVA in the inoculated microcosms. These results indicate that some wastewater microbes are able to utilize PVA for growth, and an increase in their relative abundance is shifting the community composition. Differential abundance analysis identified these microbes as belonging to the *Pseudomonas* and *Aeromonas* genera, both of which are known to degrade plastics, including PVA. The metagenomics sequencing identified two complete Pseudomonas metagenome-assembled genomes (MAG), which did not contain any known PVA degradation genes (PVA dehydrogenase and PVA hydrolase). This indicates that either these genes are present on plasmids that cannot be attributed to any specific MAG, or that other previously unknown genes are involved in PVA degradation.

These results confirm that the presence of PVA in wastewater changes it community composition, driving it towards increasing biomass and abundance of taxa that are able to utilize PVA. Moreover, the increasing CO2 production indicates that as the use of detergent pods increases, the biological oxygen demand for wastewater treatment will also increase. Alternatively, if not accounted for and remediated, the highly soluble PVA would be discharged as an effluent, where it may have impact of the aquatic life and the environment.

Gen abstract type

Poster

Section Type

AEM

264 - Biofilm Growth and Gene Regulation in Ammonia-Oxidising Bacteria (AOB)

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Although ammonia-oxidising bacteria (AOB) catalyse the rate-limiting step of nitrification, they also significantly contribute to climate change by producing nitrous oxide (N_2O), nitrite (NO_2^-), and, indirectly, nitrate (NO_3^-). N_2O is a greenhouse gas 273 times more potent than carbon dioxide, while NO_2^- and NO_3^- are pollutants that leach into water bodies, causing eutrophication. It is unsurprising, then, that several recent studies have focused on developing chemical and biological strategies to mitigate AOB-mediated N_2O emissions and nitrogen oxide leaching. However, most studies on the ecophysiology and genetic regulation of these microbes have been conducted in liquid culture, overlooking their natural tendency to grow in surface-attached biofilms and the resulting shifts in gene expression and metabolic function. In this study, we aim to investigate AOB physiology during biofilm growth and link observed phenotypic changes to shifts in gene regulation. *Nitrosospira multiformis* ATCC 25196 was chosen as a model strain for this study, as it has been extensively studied in liquid cultures. *N. multiformis* biofilm cultures will be established by immobilising cells in hydrogel beads. Subsequently, we will compare growth rates between biofilm and liquid cultures using nitrite quantification assays and gas chromatography. To quantify biofilm proliferation and EPS production in the hydrogels, fluorescence microscopy and scanning electron microscopy (SEM) will be used over several passages. Concomitant with the physiological characterisation of the AOB biofilm cultures, RNA sequencing will be used to evaluate differences in gene expression between liquid and biofilm cultures. Preliminary results demonstrate successful immobilisation of *N. multiformis* in hydrogel beads with maintained viability. We anticipate that biofilm cultures will show distinct growth kinetics and gene expression profiles compared to planktonic cultures, particularly in N_2O production pathways and extracellular polymeric substances (EPS) biosynthesis genes, based on biofilm studies in related bacteria.

Gen abstract type

Poster

Section Type

AEM

265 - Exploring Manitoba's saline springs for novel halophilic biosurfactant producers.

Nathaniel Poitras[1], Tiana Steeves[1], Marike Palmer[1]

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Biosurfactants are biologically produced alternatives to petroleum-based surfactants, which are more biodegradable, less ecotoxic, and less persistent in the environment. Some of the best-characterised biosurfactant producers belong to the opportunistic pathogenic species, *Pseudomonas aeruginosa*, however, concern regarding safety in the food, health, and cosmetic industries limits applications developed from this organism. With the prominence of surfactants in nearly all industries, the need for identifying diverse and non-pathogenic biosurfactant-producing microbes to alleviate our reliance on petroleum-based surfactants are ever growing. During a recent bacterial isolation study from the saline springs of Manitoba, specifically targeted at biosurfactant-producers, five strains putatively identified as *Aquipseudomonas* were recovered. Here, we classify and taxonomically describe these strains from their genome sequences and confirm their ability to produce biosurfactants. The five strains were initially classified using the Genome Taxonomy Database Toolkit and identified as belonging to the recently proposed genus, *Aquipseudomonas*, in the family *Pseudomonadaceae*. Two strains belonged to the species *Aquipseudomonas anguilliseptica*, while three strains represented species new to science. Phylogenomic and overall-genome-relatedness indices suggested the three novel strains represented at least two novel species. As the genomes of these strains encoded partial rhamnolipid biosynthetic pathways, these strains were screened for biosurfactant production *in vitro*. The presence of biosurfactants in cell-free supernatant was then assessed by performing oil drop collapse and oil spreading assays. All five strains showed weak to moderate surfactant activity, though strain TS-OO-06 had the greatest surfactant activity and most closely resembled the activity of commercial surfactants. With these strains being i) distinct from any known pathogens, ii) robust due to their survival in moderately extreme settings based on their isolation source, and iii) capable of biosurfactant production based on preliminary screenings, these strains show promise for further process development. Future research will entail pursuing optimization for surfactant production and characterization of produced surfactants in pursuit of a cleaner, greener solution to synthetic surfactants.

Gen abstract type

Poster

Section Type

AEM

267 - Screening, isolation, and characterization of root nodule-associated bacteria with N_2O-reducing activity in legume crops across Canada

Kyra Li[1], Yuan-Ching Tien[1], Andrew Scott[1], Wayne Calder[1], Craig Drury[1], Aga Pajak[1], Frederic Marsolais[1], Michael Fruci[1]

[1]Agriculture and Agri-Food Canada

Agriculture is a major source of nitrous oxide (N_2O), a potent greenhouse gas and major contributor to stratospheric ozone depletion. Agricultural N_2O emissions originate from nitrogen fertilized soils through microbial processes of denitrification and nitrification. Bacteria harboring the *nosZ* gene, which encodes for the nitrous oxide reductase enzyme, NosZ, have the potential to mitigate emissions by reducing N_2O into N_2. Certain rhizobia, including *Bradyrhizobium* spp. and *Ensifer* spp., possess this capability and have been shown to reduce N_2O emissions in laboratory and field studies, making them promising candidates as inoculants of leguminous crops. Here, presumptive rhizobia were isolated on Congo red plates from thousands of root nodules collected from alfalfa, fava bean, pea, pole bean, and dry bean crops grown in Saskatchewan, Manitoba, and Ontario. Isolates were screened for the *nosZ* gene using colony PCR. A total of 49 *nosZ*-positive isolates were identified and subjected to whole-genome sequencing using Oxford Nanopore long-read sequencing. Genome analysis confirmed the presence of *nosZ* and revealed the taxonomic identities of the isolates, which spanned multiple genera. The majority of isolates belonged to the genus *Pseudomonas*, with additional isolates belonging to *Ensifer*, *Pantoea*, *Rhizobium*, *Neorhizobium*, *Agrobacterium*, and *Achromobacter*. Rhizobial isolates including *Ensifer meliloti*, *Ensifer adhaerens, Rhizobium spp*., and *Neorhizobium spp*., were further assessed for their NosZ production levels and ability to reduce N_2O under anaerobic conditions. These isolates were compared with the well-studied soybean-associated N_2O-reducing rhizobia including *Bradyrhizobium diazoefficiens* USDA110 and *B. ottawaense* MIAE 01942. Collectively, these results provides insight into the presence of *nosZ*-positive bacteria associated with the root nodules of legume crops across Canada and support their potential use as inoculants to mitigate N_2O emissions.

Gen abstract type

Poster

Section Type

AEM

268 - Chlorine-Resistant Sewage Enterobacteriaceae as Potential Reservoirs of Antimicrobial Resistance: A Comparative Genomic Analysis

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Background: Chlorine-resistant *Enterobacteriaceae* in municipal wastewater may persist through disinfection and contribute to the environmental persistence and spread of antimicrobial resistance (AMR). Previous work in our group identified chlorine resistance and AMR in *Enterobacteriaceae* recovered from Alberta sewage, suggesting that these organisms may survive chlorination and act as environmental reservoirs of AMR. However, the environmental and public health relevance of these chlorine-resistant sewage isolates remains unclear, including with respect to their AMR contribution. This study used a comparative genomic approach to characterize chlorine-resistant *Enterobacter* spp., *Citrobacter* spp., and *Klebsiella oxytoca* complex (KoxC) isolates from Alberta wastewater treatment plants. This comparative approach allowed us to situate the sewage isolates within the phylogenomic context of the family *Enterobacteriaceae*, to assess relatedness across human, animal, and environmental sectors, and to identify genus-specific genomic features relevant to AMR and virulence.

Methods: Whole-genome sequences of 95 chlorine-resistant *Enterobacteriaceae* isolates from chlorinated raw sewage of 10 Alberta wastewater treatment plants were analyzed, including *Enterobacter* spp. (n = 42), *Citrobacter* spp. (n = 40), and KoxC isolates (n = 13). These genomes were compared with publicly available genomes retrieved from the National Center for Biotechnology Information (NCBI) Reference Sequence Database (RefSeq) between November 4, 2025 and January 3, 2026, representing human, animal, and environmental sources. Comparative analyses included multilocus sequence typing as well as marker-gene phylogenomics implemented in PhyloPhlAn to reconstruct core-genome phylogenetic relationships. Metadata from publicly available genomes were curated to classify isolate origin for cross-sector comparison. Resistome profiling using the Resistance Gene Identifier with the Comprehensive Antimicrobial Resistance Database was also carried out. Several further analyses are ongoing, including virulence factor gene screening using Abricate with VFDB-derived datasets and pairwise core-genome single-nucleotide polymorphism (SNP) analysis, to further resolve fine-scale genomic relatedness.

Results: The three genera showed distinct population structures. Compared with 95 publicly available NCBI RefSeq genomes, the Alberta sewage *Enterobacter* isolates formed several distinct clusters, including an ST-32-enriched clade, while remaining phylogenetically linked to genomes from multiple sectors. Their resistomes showed a shared core profile with additional cluster-level variation, marked by predominance of the *blaACT* group. Compared with 126 publicly available NCBI RefSeq genomes, *Citrobacter* isolates were distributed across multiple lineages, with several small wastewater-associated subclusters but no single Alberta-specific clade; their resistomes were characterized by the *blaCMY* group and conserved *arnT*/*pmrF*, with limited additional variation. Compared with 125 publicly available NCBI RefSeq genomes, KoxC isolates were also dispersed across multiple lineages rather than forming a wastewater-restricted clade and displayed a highly conserved resistome dominated by *arnT*/*pmrF,* multidrug efflux/regulatory determinants, and *fosA5*. Pairwise core-genome SNP analysis and putative virulence factor gene screening are ongoing to further help interpret relatedness between wastewater isolates and publicly available genomes from human, animal, and environmental sources.

Conclusion: Chlorine-resistant sewage *Enterobacteriaceae* showed clear genus-level differences in population structure relative to publicly available genomes and in resistome composition. These findings support the value of comparative genomic surveillance of wastewater-associated *Enterobacteriaceae* for identifying environmentally persistent lineages and informing AMR monitoring in engineered water systems.

Gen abstract type Poster

Section Type AEM

269 - Impacts of prolonged liquid culture on growth and soil performance across diverse bacterial isolates

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Microbial inoculants are widely used in agriculture to promote plant growth, increase stress tolerance, and improve soil health. The global demand for agricultural bioinoculants has been rising, driven by the need for sustainable farming practices. To produce probiotics in high quantities for commercialization, microbes are incubated for prolonged periods in liquid culture, commonly tryptic soy broth (TSB). However, formulation is challenging because a controlled laboratory environment imposes selective pressures different from the variable conditions of soil. Thus, microbial adaptation to media may lead to unreliable soil survival and may not be beneficial after reintroduction to their natural environment. This discrepancy on microbial performance remains an unknown barrier to effective application across diverse soils. Moreover, there is a growing need to include diverse isolates in experimental evolution work, as most studies have been done on a few model organisms such as *E. coli*. This study investigates how prolonged growth in TSB affects the in-soil performance of diverse bacterial isolates. A total of 14 isolates from 7 genera will be cultured over 45 days and passaged every 72 hours. Here, we used 2 commercial isolates to compare fitness observations between naturally occurring soil isolates and isolates that are commercially sold as industry standards. At defined timepoints, in-culture growth will be quantified via optical density and colony-forming units, while in-soil survival, proliferation, metabolic activity, and drought-cycle stress resilience will be assessed using biotically cleared microcosms. This research characterizes the temporal dynamics of in-soil fitness loss and the variation in resilience across diverse isolates. In addition, revealing evolutionary potential and constraints can be used to address a gap in microbial inoculant development and reduce chemical input dependency.

Gen abstract type

Poster

Section Type

AEM

273 - Encapsulated Tryptophan to Enhance Protective Indoles in the Weaning Transition in Piglets

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In modern pig production systems, early weaning occurs when piglets are three to four weeks of age. This abrupt and early transition induces weaning stress that can disrupt gut integrity and homeostasis and cause shifts in microbial composition, consequently increasing the risk of pathogen growth, diarrhea, and mortality. Therefore, nutritional interventions that support intestinal development, gut microbiome health, and integrity is crucial for maintaining animal health and productivity. Tryptophan is an essential amino acid necessary for animal growth and its microbial metabolism by commensal bacteria in the gut results in the production of beneficial indoles. Indoles support the immune system and gut integrity through activation of aryl hydrocarbon receptors. As a result, the goal of this study is to investigate the impact of tryptophan on gut integrity and morphology. This study is designed in two steps using encapsulated forms of tryptophan. The key aspect of this study is the targeted delivery of the tryptophan amino acid to the distal parts of the colon using inulin-based microcapsules. These microcapsules provide the opportunity for tryptophan to pass through the small intestine and be metabolized by commensal microbes residing in the large intestine. As microbial diversity and abundance are higher in the distal colon, we expect that more indoles will be produced under these conditions, which may reduce intestinal permeability and inflammation, thereby mitigating weaning transition stress. As a result, in the first experiment, piglets will be fed with a basal diet containing different levels of 0.1%, 0.2%, and 0.4% of encapsulated tryptophan to optimize and quantify indole production at different inclusion levels. In the second experiment, the effect of selected encapsulated tryptophan on intestinal permeability, inflammation, and microbial community will be tested. We expect that the results of this experiment will provide insight on the optimal dosage of encapsulated tryptophan to be used as feed additives as well as the beneficial effect of this product on pig health.

Gen abstract type

Poster

Section Type

AEM

275 - A Metabolomics-Guided Strategy Identifies LAB-Derived Compounds with Potent Anti-*Helicobacter pylori* Activity

Jean Wiliane Marbou Takougoum[1], C. Kang[1], D. Carroll[1], M. Hildebrandt[1], J. Auger[2], S. Bronner[2], S. Binda[2], C. Creuzenet[1]

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Helicobacter pylori (HP) colonizes 50% of the world population and can cause gastric ulcers and cancers. Its increasing antimicrobial resistance calls for new biotherapeutics. We screened 25 Lactic Acid Bacteria (LAB) and showed that 5 (L21-L25) secrete molecules that inhibit HP's growth and virulence. As such, LAB secretions are potential sources of anti-HP agents which could be used as antibiotic complements. To investigate potential role of metabolites in the effect, all the LAB secretions were analyzed by MS spectrometry. 999 metabolites were identified. After categorizing the samples in active versus inactive groups based on prior phenotypic analyses, machine learning was used to rank the compounds according to their correlation with activity. Further, the top metabolite candidates showing higher abundance in a specific LAB strain were selected for experimental validation based on prior strain-specific phenotypic data. The 10 candidates chosen and strains that produce them are: L-10 (L22;), 11 (L24), 58 (L21, L22, L23, L25), 140 (L24;), 169 (L24, L25;), 186 (L24;), 196 (L24), 201 (L24), 267 (L24), and 310 (L24). Two more metabolites were selected based on their absence in the non-active group: 37 (L23) and 6 (L24). The 6 candidates tested so far in a 24h anti-HP susceptibility assay at neutral pH, with or without urea, showed significant inhibitory effects on HP, with minimal bactericidal concentrations ranging from 0.4 mM to 50.0 mM, compound 169 being the most potent. The anti-HP activities of all tested compounds were maintained in the presence of urea, which is important since HP resides in urea-rich stomach environment. Their effects on HP virulence factors, pH and potentiation of antibiotics are under investigation. So far, some compounds partly recapitulate the down regulation effects seen on urease activity with complete LAB secretions.

Further, as L25 is the major constituent of Lacidofil® which increases the rate of HP eradication in conjunction with standard therapy in clinical trials, we characterized the anti-HP effects of Lacidofil *in vitro*. We showed that Lacidofil® secretions decrease HP's viability and pro-inflammatory activity as effectively as L25 secretions.

This study provides alternative approaches to identify anti-HP agents, validating that LAB secrete metabolites with anti-HP activity and supporting their application as adjuvants or complements to anti-HP therapies.

Gen abstract type

Poster

Section Type

AEM

277 - Synthetic Peptides as Recognition Elements for Bacterial Detection

Mustafa Al-gafari[1], Houman Moteshareie[2], Thomas Kazmirchuk[1], Jiashu Wang[1], Emma Vidal-Tan[1], Stéphane Bernatchez[3], Valar Anoop[3], Ashkan Golshani[1], Azam Tayabali[2]

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Microbial detection is essential for biotechnology applications, environmental monitoring, and public health surveillance. Antibody-based systems remain widely used for microbial identification; however, their development is often costly, time-intensive, and reliant on animal immunization. Synthetic peptides provide a promising alternative because they can be designed *in silico*, synthesized rapidly, and adapted to a range of detection platforms. In addition to offering practical advantages in production and scalability, peptide-based probes may provide a more flexible and ethically sustainable strategy for developing next-generation microbial detection tools. In this study, we investigated the potential of synthetic peptides to function as alternatives to antibodies for the detection of bacterial species relevant to biotechnology oversight and microbial monitoring.

This work focused on bacterial species of regulatory and applied interest, including *Bacillus subtilis*, *Priestia megaterium*, *Pseudomonas synxantha*, and pathogenic *Pseudomonas aeruginosa*. Candidate peptides were generated using the In-Silico Protein Synthesizer (InSiPS), an AI-based design platform that enables peptide selection against defined target proteins while screening against non-targets to improve specificity. Peptides with the strongest predicted performance were synthesized and evaluated experimentally for binding to whole bacterial cells. Initial validation was carried out using peptide-based binding assays to assess recognition of target organisms and to compare signal intensity relative to non-target controls.

Our results support the feasibility of using synthetic peptides affimers as microbial recognition elements. Selected peptides demonstrated measurable binding to bacterial cells, with stronger signal observed for intended targets than for negative controls. These findings indicate that peptide probes can distinguish bacteria at the whole-cell level and support their use in practical detection workflows. Building on these results, validated peptides were adapted to bead-based detection using Luminex Bio-Plex technology. Although a fully multiplexed format remains under development, initial bead-based experiments have established proof of concept for peptide mediated bacterial detection in a single-plex configuration. This represents an important step toward the future development of multiplex assays capable of detecting multiple bacterial species simultaneously in mixed samples.

Overall, our findings support synthetic peptides as promising alternatives to antibodies for bacterial detection. Compared with conventional antibody generation, peptide probes offer advantages in design flexibility, speed of production, scalability, and potential cost reduction, while avoiding dependence on animal-based immunization. With further optimization, peptide-based bead assays may enable robust multiplex detection platforms with applications in biotechnology monitoring, environmental surveillance, and public health testing.

Gen abstract type

Poster

Section Type

AEM

281 - Determinants of Bacteroidota colonization in broiler chickens: the role of host origin

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Early-life microbial colonization is critical for gut development and long-term health in broiler chickens; however, modern intensive production systems limit exposure to host-adapted obligate anaerobes such as members of the phylum Bacteroidota. The determinants governing successful establishment of these microbes in the avian gastrointestinal tract remain poorly understood. This study investigated whether host origin influences colonization efficiency of Bacteroidota in broiler chickens. Day-old broiler chicks (n = 63) were housed in individually ventilated isolators and inoculated via oral gavage with either chicken-derived, human-derived, or mixed Bacteroidota communities. Colonization dynamics were assessed using 16S rRNA amplicon sequencing and quantitative PCR targeting Bacteroidota, Enterobacteriaceae, and total bacterial abundance. Oral gavage resulted in successful early establishment of Bacteroidota in the gastrointestinal tract, with increased relative and absolute abundance observed by Day 14 ($p < 0.01$). Both chicken- and human-derived strains were able to colonize the chicken gut; however, chicken-derived strains demonstrated greater persistence and colonization efficiency, particularly under competitive conditions, suggesting that host origin contributes to colonization success in the avian gut environment. To explore potential mechanisms underlying these differences, representative isolates are currently being evaluated for oxygen sensitivity. Enhanced colonization was associated with increased total bacterial abundance and improved early growth performance ($p < 0.01$). Overall, these findings suggest that host origin is a key factor influencing successful establishment of Bacteroidota in broiler chickens, with oxygen sensitivity likely contributing as an additional determinant. Understanding these factors provides insight into mechanisms governing early-life gut microbial assembly and supports the development of targeted microbial strategies to improve poultry health and production efficiency.

Gen abstract type

Poster

Section Type

AEM

288 - Strain level diversity of *Brettanomyces bruxellensis* in spontaneous beer fermentations: Does it matter?

Maanasa Mudoor Sooresh[1], Shane Carey[1], Victor Loegler[2], Anne Friedrich[2], Joseph Schacherer[2], Ben Bourrie[3], Benjamin Willing[1]

[1]University of Alberta, [2]University of Strasbourg, [3]Macewan University

Lambic style spontaneous beer production has been largely limited to Belgium over the past few centuries with a stable brewhouse microbiota. A new lambic style brewery has been established in Canada, and there is a need to evaluate the microbial communities in Canadian spontaneous beer that use wooden wine barrels to mimic the lambic beer production. Particularly, *Brettanomyces bruxellensis,* often associated with wooden barrels, is a yeast species that is critical to the fermentation and maturation of lambic beers. There is evidence of loss of genes in lambic beer strains which makes them functionally different from their wine counterparts. There is insufficient representation of spontaneous beer strains, with few spontaneous beer isolates from outside of Belgium. In this study, we sampled across 3 barrels from a Canadian spontaneous beer facility to isolate *B. bruxellensis* strains and understand the genetic diversity prevalent. 8 strains were selected based on randomly amplified polymorphic DNA (RAPD) banding patterns and the genomes were sequenced. SNP analysis showed that all the strains were diploid and heterozygous, however placement of the 8 strains relative to 1060 strains in neighbour joining tree based on the study conducted by Loegler et al., 2025 were found to be at three different positions within the diploid clade. Functional gene analysis revealed that the 20 genes missing in previously studied lambic beer strain ST05.12/22a were present in the strains used for this study. Moreover, heterogeneity of the β-glucosidase gene was revealed among strains found within the same barrel. This study provides an insight into the genetic similarities and differences prevalent in *Brettanomyces bruxellensis* strains isolated from Canadian spontaneous beer fermentations.

Gen abstract type

Poster

Section Type

AEM

289 - Phage Discovery in Methanotrophs: A Novel Approach to Methane Mitigation and Bio-Conversion

Ismailcan Bayrakceken[1], Dominic Sauvageau[2], Lisa Stein[1]

[1]Department of Biological Sciences, University of Alberta, Edmonton, Canada, [2]Department of Chemical and Materials Engineering, University of Alberta, Edmonton, Canada

The discovery of lytic phages that infect methane-oxidizing bacteria (MOB) may provide insight into phage diversity, phage-host specificity, molecular mechanisms and their impact on the physiology and ecology of their host. The MOB strains used in this study are *Methylomicrobium album* (BG8), *Methylocystis sp.* (Rockwell), *Methylosinus trichosporium* (OB3b) and *Methylomonas denitrificans* (FJG1), which belong to Alpha- and Gammaproteobacteria. The current efforts are underway for isolating lytic phages targeting MOB from environmental samples such as floating island soils, compost, wastewater, and activated sludge. These samples are chosen because they support dense MOB communities and are expected to contain associated viral strains. The collected samples are filtered through cellulose acetate filters, removing contaminants while allowing phages to pass through, creating phage-containing filtrates. These phage-containing filtrates are later used to infect MOB cultures, which are then incubated and monitored over time using optical density measurements. The cultures showing reduced growth suggest lytic activity due to phage infection from the filtrate. Further confirmation of bacteriophage is done through performing transmission electron microscopy (TEM) on selected samples that show lytic phage activity. Following this, DNA/RNA is extracted from phage-containing filters, and sequencing is conducted to enable phylogenetic placement and characterization of viral diversity. Current experiments have shown promising results, with one environmental sample demonstrating reduced growth in MOB cultures and successful genetic material extraction from concentrated phage filtrate. Thus the findings are supporting the presence of active lytic phages. Overall, this work aims to improve the understanding of phage diversity and virus-host interactions in methanotrophic systems.

Gen abstract type

Poster

Section Type

AEM

290 - Divergent branchial nitrogen cycling strategies in invasive and native decapod crustaceans: ammonia oxidation versus assimilation

Brittany Sauter[1], Danielle Tarabishi[1], Tamzin A. Blewett[1], Lisa Y. Stein[1]

[1]University of Alberta

Coastal marine ecosystems are rapidly shifting under climate change, with warming, nutrient enrichment, and acidification reshaping nitrogen cycling at the interface of host and environment. Decapod crustacean gills are multifunctional organs that mediate gas exchange, ion regulation, and nitrogenous waste excretion, making them a critical but understudied niche for nitrogen-cycling microbial communities. The European green crab, *Carcinus maenas*, is an established invasive species along the Northeast Pacific and shows broad physiological tolerance compared to co-occurring native crabs. Whether this invasion success is reflected in a functionally distinct gill microbiome remains unknown.

We characterized the gill microbiomes of invasive *C. maenas* and three sympatric native crabs, *Cancer productus*, *Metacarcinus magister*, and *Metacarcinus gracilis*, collected from Bamfield, British Columbia. Anterior gills 4 and 5 and posterior gill 8 were sampled across two independent field seasons. We paired 16S rRNA V3-V4 amplicon sequencing with qPCR targeting nitrogen-cycling functional genes: *amoA* of ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and complete ammonia oxidizers (comammox), *nxrB* of nitrite-oxidizing *Nitrospira*, and *ureC* of ureolytic prokaryotes.

Community structure differed significantly by species and invasion status at all three gill positions (PERMANOVA, $p < 0.01$), with species identity explaining 44.7 to 50.4% of the variation. At posterior gill 8, alpha diversity diverged significantly among species, with *C. maenas* showing higher Shannon diversity than *M. magister*. Taxonomic composition at gill 8 revealed a striking functional signal: *C. maenas* gills harboured detectable *Nitrosomonas* (AOB) and *Nitrospira*(NOB), taxa that were absent or at trace levels in all native species, alongside a novel dominant genus *Fuerstia*. Native crabs were instead dominated by *Pseudahrensia* and *Robiginitomaculum*, with *M. magister* uniquely enriched in *Albimonas* and *Aminobacter*.

qPCR quantification supported these compositional patterns with clear functional divergence. *C. maenas* gill 8 returned the highest AOB *amoA* abundances in the dataset (up to 148 copies ng^{-1} DNA), one to two orders of magnitude above native species. In contrast, *nxrB* and comammox *amoA* abundances were highest in native crabs, with *M. magister*reaching over 17,000 *nxrB* copies ng^{-1} DNA. *ureC* was most abundant in *M. magister* gill 8, consistent with its known reliance on urea cycling during postprandial ammonia accumulation.

Together, these results reveal that invasive and native decapods host functionally divergent gill microbiomes, with *C. maenas* uniquely enriched in ammonia-oxidizing taxa while native crabs retain nitrite-oxidizing and ureolytic capacity. This partitioning of branchial nitrogen cycling suggests that gill-associated microbes may contribute to the physiological plasticity underlying *C. maenas* invasion success and highlights the gill microbiome as a functional axis on which invasive and native species diverge in Pacific Northwest coastal habitats.

Gen abstract type

Poster

Section Type

AEM

291 - Bovine respiratory microbiota transplantation alters nasopharyngeal bacterial diversity and structure in feedlot cattle

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Bovine respiratory disease is the most prevalent health concern in feedlot cattle, accounting for significant economic losses and metaphylatic antimicrobial use. The disease is multifactorial but typically associated with infection by *Histophilus somni*, *Mannheimia haemolytica*, *Mycoplasma bovis*, and *Pasteurella multocida* bacterial pathogens. Stressors related to cattle production, such as abrupt weaning, transportation, and comingling in a feedlot, can cause disturbance of the natural upper respiratory tract microbiota. This increases susceptibility to pathogen proliferation, and lung translocation and infection. Stabilizing the upper respiratory microbiota may therefore reduce pathogen colonization and growth that takes place after feedlot entry. In this study, the effect of respiratory microbiota transplantation on the nasopharyngeal bacteria of feedlot calves was evaluated. Nasal discharge from mature cows in an isolated disease-free herd was used as donor inoculant in a feedlot study. The treatments were (n =16 per treatment) : i) “MICRO”: intranasal microbiota on day 1, ii) “CON”: intranasal saline on day 1, iii) “ABX+MICRO”: antibiotic injection on day 1 & intranasal microbiota on day 11, iv) “ABX+CON”: antibiotic injection on day 1 & intranasal saline on day 11.

The nasopharynx of study calves was sampled by swab at feedlot entry, before treatment application, and periodically up to 35 days after entry. Swabs were processed for DNA extraction and the 16S rRNA gene was sequenced to evaluate bacteria. The alpha diversity metrics Shannon diversity index ($P<0.001$) and richness (observed taxa; $P<0.001$) were found to be significant with respect to time and treatment. Only Shannon diversity was affected by the interaction between time and treatment ($P<0.001$). After microbiota transplant in the ABX+microbiota treatment group, richness was increased compared to ABX treatment calves, reflecting bacterial introduction by the donor inoculant. A similar increase was not observed in the microbiota group. Within the 16S rRNA dataset, 18 phyla were identified, with the most abundant being: Pseudomonadota, 52.74%; Bacillota, 36.12; Actinomycetota, 4.54%; Bacteroidota, 2.99%; Fusobacteriota, 0.94; Deinococcota, 0.94%; Deinococcota, 0.82%; Chloroflexota, 0.24; and Methanobacteriota, 0.14%. PERMANOVA revealed that microbiota structure was affected by treatment ($R2 = 0.06342$, $P= 1e-04$), sampling day ($R2 = 0.07731$, $P = 1e-04$), and interaction of treatment and time ($R2 = 0.18292$, $P = 1e-04$). Reduced variation in microbial structure was observed in calves administered the donor inoculant. Notably, RMT treatment with microbiota also reduced the abundance of *Histophilus* in groups with and without antibiotic treatment. This pilot study demonstrated the ability of respiratory microbiota transplantation to alter the nasopharyngeal microbiota of calves, potentially stabilizing respiratory bacteria after feedlot placement. Further evaluation is warranted to determine if RMT can be used to enhance respiratory health of calves and subsequently reduce antimicrobial use for BRD prevention.

Gen abstract type Poster

Section Type AEM

293 - Bioengineered *E. coli* secretes RNA-loaded outer membrane vesicles for cancer therapy

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Background: RNA-based therapeutics, including siRNA and shRNA, offer high target specificity and reduced side effects by silencing target genes in a sequence-specific manner. Despite their promise for cancer treatment, efficient delivery remains a challenge due to their instability and poor cell permeability. Current RNA delivery systems, such as lipid nanoparticles, often require complex, environmentally sensitive preparations that can be costly. Therefore, it is essential to develop a simpler and more efficient RNA delivery system. In this study, we applied synthetic biology approaches to develop and improve an RNA delivery platform using the bioengineered *E. coli* that continuously secretes RNA-loaded outer membrane vesicles (OMVs) for potential cancer therapy.

Methods: To enhance the OMV production, we introduced an *nlpI* deletion into *E. coli* HT115 strain by P1 phage transduction. Synthetic DNA constructs encoding siRNA or shRNA were designed and introduced into the *E. coli* HT115 ΔnlpI strain. OMVs were isolated from bacterial cultures by ultracentrifugation, and RNA expression and OMV packaging were analyzed via RT-PCR using RNA extracted from OMVs. To improve shRNAs loading into OMVs, we constructed an *E. coli* HT115 ΔnlpI system expressing shRNA fused to one or three MS2 hairpin RNA motifs (shRNA-MS2 or shRNA-(3x)MS2) and a MS2 binding protein fused to maltose binding protein (MBP-MS2BP), which is a periplasmic protein natively expressed in *E. coli*. This design was intended to promote RNA/protein complex formation and enhance shRNA packaging into OMVs. shRNA packaging efficiency was evaluated by RT-qPCR on RNA extracted from equal numbers of OMVs and compared with control OMVs from HT115 ΔnlpI expressing shRNA-(3x)MS2 alone.

Results: Deletion of *nlpI* in *E. coli* HT115 significantly increased OMV production, yielding more than twice as many OMVs as the wild-type strain. RT-PCR confirmed siRNA expression and successful packaging into OMVs, with higher secretion levels of smaller siRNA fragments. RT-qPCR showed that OMVs from the HT115 ΔnlpI / pMBP-MS2BP-shRNA-(3x)MS2 contained a significantly higher level of shRNA relative to housekeeping RNA than OMVs from HT115 ΔnlpI / pshRNA-(3x)MS2 group ($p < 0.0001$).

Conclusion: Our proposed *E. coli* RNA delivery platform enables siRNA/shRNA expression and packaging into OMVs, presenting a potential low-cost delivery system for RNA-based cancer therapy. Our synthetic biology strategy improves the shRNA loading into OMVs and supports the development of engineered bacteria as therapeutic RNA delivery vehicles.

Gen abstract type

Poster

Section Type

AEM

295 - Linking Microbial Dynamics to Treatment Efficacy in Constructed Wetlands for OSPW Remediation

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The management of industrial wastewaters represents a critical global challenge requiring sustainable, low-energy solutions that reduce contaminant loads while restoring ecological function. In Alberta, Canada, bitumen extraction from the Athabasca oil sands has generated vast volumes of oil sands process-affected water (OSPW), a complex mixture containing salts, metals, and persistent organic contaminants such as naphthenic acid fraction compounds (NAFCs). These compounds pose risks to aquatic ecosystems and necessitate scalable remediation strategies. Constructed wetland treatment systems (CWTS) are a promising nature-based approach that leverages plant-microbe-substrate interactions to attenuate contaminants; however, their optimization depends on a mechanistic understanding of microbial community structure and function.

This study, conducted as part of the Genomics Research for Optimization of Constructed Treatment Wetlands project, investigates how substrate type and refill of fresh OSPW influence microbial community dynamics and NAFC attenuation in mesocosm-scale CWTS. Greenhouse mesocosms planted with *Carex aquatilis* were established using either peat or compost as the organic substrate over a layer of sand with OSPW continuously circulating in a surface-flow closed-loop system. After 40 days, half of the mesocosms were drained and refilled with fresh OSPW to simulate episodic contaminant loading. Over 77 days, both systems achieved substantial NAFC removal (~50-70%), with compost-based mesocosms demonstrating consistently greater attenuation and enhanced plant growth relative to peat systems. Moreover, compost-based systems showed slightly higher NAFC removal rates following the refill event. Microbial communities in both water and sediment were characteristic of CWTS and included taxa with putative roles in hydrocarbon degradation. Metagenomic analyses revealed that substrate type was the primary driver of structural community composition. These structural communities remained stable following the refilling event, indicating resilience to episodic contaminant loading. In contrast, metatranscriptomic analyses revealed dynamic, substrate-dependent shifts in active microbial communities. Functional diversity increased significantly over time in peat systems, particularly following refilling, whereas compost systems maintained a comparatively stable transcriptional profile. Refilling altered active community structure without disrupting substrate-defined clustering, indicating that functional trajectories remained constrained by substrate. Despite increased functional diversity, peat systems did not achieve greater NAFC removal; instead, compost systems consistently exhibited higher attenuation, suggesting that a more stable and functionally consistent microbial community may better support sustained contaminant removal under these conditions.

Together, these findings demonstrate that substrate selection governs both the stability and functional dynamics of microbial communities in CWTS, with direct implications for contaminant attenuation. By linking microbial structure and activity to treatment performance under realistic disturbance scenarios, this work advances the optimization of CWTS for large-scale OSPW remediation and contributes to the broader application of microbial systems in addressing global water quality challenges.

Gen abstract type Poster

Section Type AEM

296 - Characterization of Extraintestinal Pathogenic Escherichia coli and its Antimicrobial Resistance in Stormwater Reservoirs from Alberta

Kate Edwards[1], Kanghee Ryu[1], Candis Scott[1, 2], Markus Gänzle[1, 2, 3], Norman Neumann[1, 2], Simon Otto[1, 2, 3, 4]

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Background: Extraintestinal pathogenic *Escherichia coli* (ExPEC) can survive wastewater treatment and enter surrounding water environments through discharge of treated effluents. Their ability to survive treatment is facilitated by phenotypic tolerance to heat, cold, chlorine, nutrient deprivation, and osmotic stress. These effluent-derived strains often share antimicrobial resistance (AMR) and virulence profiles with human clinical isolates. Some avian- and poultry-associated ExPEC even show close genetic similarity to human isolates, suggesting ExPEC may have zoonotic potential. However, our understanding of the presence and characteristics of ExPEC in stormwater remains unclear. Stormwater is a potential driver of AMR dissemination as it receives human and animal fecal inputs from runoff and sewer infrastructure failures. ExPEC and related AMR in stormwater may represent an important One Health threat as it may serve as a pathway for ExPEC transmission to new hosts and the transfer of AMR genes to other bacteria, contributing to a broader environmental resistome.

Objective: to explore whether pathogenic and drug-resistant ExPEC are present in stormwater reservoirs in Alberta and to assess potential risks they pose to One Health sectors.

Methods and Results: 44 initial *E. coli* isolates were collected from stormwater reservoirs in Alberta known to be impacted by human sewage, and identified using biochemical and molecular methods. Clonal relatedness was evaluated using repetitive element sequence-based PCR, which revealed 30 unique banding patterns. Isolates were screened for seven ExPEC-associated virulence genes, and 28 of 44 carried at least three virulence markers. Antimicrobial susceptibility testing revealed low overall resistance. 3 of the 44 isolates showed resistance to fluoroquinolones (including one ExPEC). Two distinct ExPEC isolates were resistant to β-lactams, one of which showed resistance to multiple third-generation cephalosporins (cefpodoxime, cefovecin, ceftazidime) and intermediate resistance to ceftiofur.

Significance: these findings suggest that stormwater reservoirs may contain presumptive ExPEC strains and with occasional antimicrobial resistance. Ongoing antimicrobial susceptibility testing using population-level screening will help to further characterize AMR profiles of stormwater ExPEC, and improve detection of rare resistant strains that are missed at the isolate level. Future genomic analysis will compare these isolates with human, animal, and environmental *E. coli* to evaluate similarities in virulence gene and antimicrobial resistance gene profiles, as well as phylogenetic relatedness, to better understand their pathogenicity and zoonotic potential. This work will advance understanding of AMR dissemination in urban water systems and guide future surveillance and programs to understand the One Health risks from environmental AMR.

Gen abstract type Poster **Section Type** AEM

298 - Local Allies or Packaged Partners? Evaluating Inoculant Effectiveness of Commercial vs. Native Arbuscular Mycorrhizal Fungi in Rough Fescue Under Greenhouse Conditions

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Grasslands dominated by Rough Fescue (*Festuca campestris*) are some of the most productive in Canada. As effective reclamation is vital to preserving their ecological integrity and function, Arbuscular Mycorrhizal Fungi (AMF) are a potential solution as it forms a symbiotic relationships with over 80% of land plants, commonly improving nutrient uptake, soil stabilization and carbon sequestration. Yet, the effectiveness between the commercial inoculants and native inoculants from the site, remains unclear in the greenhouse settings, where plants are being grown as plugs for field transplanting.

F. campestris was inoculated with native or commercial probiotic treatments and then assessed through characterizing the effects of Arbuscular Mycorrhizal Fungi on the overall growth and performance of Rough Fescue under controlled greenhouse conditions.

Methods consisted of a completely randomized greenhouse experiment using native seeds, hand-collected from the Cervo 2 site, near the Leitch Collieries Provincial Historic Site, Crowsnest Pass, AB. Four treatments were applied: Dynomyco (commercial AMF inoculant), Negative Control (no AMF), Positive Control (Promix HD growing media with AMF), and Topsoil (native AMF community).

The seedlings were germinated at the Mycology Laboratory, then transferred into ROOTRAINERS® and monitored for 16 weeks under controlled greenhouse conditions until ready for field transplanting. Weekly measurements included: Number of plant leaves, plant height, soil temperature, soil moisture and soil pH. A subsample of the grasses was harvested to measure fresh weight, biomass, root length, plant height and number of leaves. Roots were stained with ink and vinegar, to be examined under the stereo microscope for AMF colonization and under the compound microscope for AMF structures, using a gridline method.

Using JMP Pro 17 (SAS Institute Inc., Cary, NC, USA), no differences were seen in plant height or number of leaves. Root fresh weight differed among treatments, with the Positive Control producing the highest (1.604 g) mean compared with Dynomyco (0.645 g) and Topsoil (0.397 g). Root length was significantly different with Dynomyco yielding the longest roots (16.375 cm) compared with all other treatments (~12 cm).

Under non-stressed greenhouse conditions, the results were contrary to the initial prediction, Negative and Positive controls produced the highest root biomass, whereas the commercial and native treatment had the lowest. This suggests a possible carbon-cost trade-off and intraspecific competition in the native treatment where the fungi were more developed. The effects of AMF on these grasses remain to be assessed further in the field, where there could be benefits from the AMF association on plant establishment and survival on degraded or drought-prone sites.

These findings highlight the requirement to be cognizant of plant composition and growth strategies. Future studies in Rough Fescue will assess plant performance for two more consecutive years in the Crowsnest Pass within varying conditions and expanding to more AMF inoculants and beneficial bacteria. Additionally, this research is relevant to other Alberta's native grasses, forbs and shrubs within plant communities to gain further insight and refine grassland restoration practices in the future.

Gen abstract type

Poster

Section Type

AEM

299 - Novel antimicrobial interventions against *Clostridium estertheticum* spores in beef systems: implications for shelf life and food waste reduction

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Blown pack spoilage (BPS) of vacuum-packaged beef, primarily caused by the psychrotrophic spore-forming bacterium *Clostridium estertheticum*, remains a major challenge for the beef industry because it shortens shelf life, reduces product value, and contributes to food waste. In the context of the global challenge of reducing microbial spoilage and improving food system sustainability, this study evaluated the efficacy of peroxyacetic acid (PAA), ozone nanobubble solution (ONB), and plasma-activated water (PAW) against *C. estertheticum* spores in suspension, on inoculated beef, and during refrigerated storage of vacuum-packaged beef. In aqueous suspension, treatment efficacy depended strongly on matrix and contact time, with PAA showing the greatest sporicidal activity and achieving up to approximately 2 log CFU/mL reduction after 10 min in water, whereas ONB and PAW showed minimal inactivation. In a more practical meat immersion system, ONB gave the greatest reduction, reaching approximately 1 log CFU/cm² at 10 min, followed by PAA at about 0.7 log CFU/cm², while PAW did not differ meaningfully from water washing alone. A 6-week shelf-life study at 4°C showed that *C. estertheticum* populations increased rapidly from week 0 to week 2, then plateaued, and none of the antimicrobial treatments significantly suppressed outgrowth relative to untreated controls. Total aerobic counts also increased over time, particularly in fat tissue, indicating that meat composition had a stronger influence on microbial proliferation than treatment. 16S rRNA gene amplicon sequencing further showed that storage time was the dominant driver of microbiota succession, with declining alpha diversity and convergence toward *Clostridium*-dominated communities regardless of intervention. Quality assessments showed limited treatment effects on pH and sensory deterioration overall, although lipid oxidation was more variable in fat tissue and was greatest in ONB-treated samples at later storage stages. Together, these findings demonstrate that while oxidative antimicrobial interventions can reduce spores under controlled conditions, their efficacy is markedly constrained in meat systems. Effective control of psychrotrophic clostridial spoilage in vacuum-packaged beef will likely require optimized multi-hurdle strategies that address both microbial resistance and matrix protection, thereby helping reduce spoilage-associated food losses.

Gen abstract type

Poster

Section Type

AEM

302 - A tea-riffic indicator: the Labrador tea microbiome and boreal ecosystem health

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Labrador tea (*Rhododendron groenlandicum)* is an evergreen shrub that continues to be foraged by Canada's Indigenous Peoples as traditional medicine. The Canadian boreal forest, where it grows, is abundant in natural resources such as lumber, which is harvested through industrial means. Anthropogenic disturbances associated with these industrial operations have raised concerns from residing Indigenous communities over the health of the boreal ecosystem and the safe consumption of foraged traditional foods and medicines, including Labrador tea. Our study aims to use the microbiome of Labrador tea to assess the impact of such anthropogenic activities as a proxy for boreal ecosystem health. Hence, we first **characterized and defined the core Labrador tea microbiome, then compared changes in this core microbiome across a forest disturbance gradient.**

In collaboration with the Bigstone Cree Nation, three Labrador tea specimens were collected each spring and autumn over two years, across seven sites ranging from low (relatively undisturbed) to high forest disturbance (clear-cut blocks sprayed with herbicides). The microbial communities of various above-ground (phyllosphere) and below-ground (rhizosphere) plant organs were characterized by sequencing the V4 region of the 16S rRNA gene.

We found that the phyllosphere and rhizosphere communities were statistically distinct, with higher relative abundances of *Hymenobacteraceae, Beijerinckiaceae,* and *Sphingomonadaceae* in the phyllosphere, and of *Acidimicrobiaceae*, *Dongiaceae,* and *Gemmatimonadaceae* in the rhizosphere. While the community composition of rhizosphere plant organs (roots and rhizome) was not different, the communities of phyllosphere plant organs (leaf and stem) were statistically distinct. Hence, we characterized the core microbiome as the taxa that remained consistent within three distinct microhabitats: the phylloplane (leaf), cauloplane (stem), and rhizosphere, across all seasons for plants collected from low-disturbance sites. The core microbiome consisted of ASVs primarily belonging to *Pseudomonadaceae* in the phylloplane, *Sphingomonadaceae* and *Acetobacteraceae* in the cauloplane, and *Burkholderiaceae*, *Mycobacteriaceae* and *Xanthobacteraceae* in the rhizosphere.

When compared across disturbance levels, plants from low- and high-disturbance sites had significantly distinct microbial communities. Importantly, taxa belonging to the *Pseudomonadaceae* family were in lower relative abundance across all organs of the plants sampled from high-disturbance sites. Moreover, the cauloplane and phylloplane of plants from high-disturbance sites had a higher relative abundance of *Hymenobacteraceae*, whereas the rhizosphere had a higher relative abundance of *Mycobacteriaceae*.

Our research presents the first comprehensive study of the Labrador tea microbiome, showing that forest disturbances affect it both above and below ground. Overall, these demonstrate that changes in the microbiome of Labrador tea, a culturally important species, can be used as a proxy for boreal ecosystem health.

Gen abstract type Poster

Section Type AEM

304 - One Panel, Many Pathogens: Pan-Viral Wastewater Genomics Across Alberta Communities

Linnet Immaraj[1], Qiang Jiang[1], Fan Zhao[1], Judy Qiu[1,2], Tiejun Gao[1], Matthew Croxen[1,2], Michael Parkins[3], Casey Hubert[3], Bonita Lee[1], Xiaoli Pang[1]

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Wastewater-based epidemiology is a powerful complementary tool to clinical surveillance by providing population-level, non-invasive monitoring of infectious disease activity. However, wastewater monitoring often relies on targeted qPCR assays for predefined viruses, limiting detection of broader virome changes, co-circulating pathogens, and unusual or re-emerging threats. As respiratory, enteric, and vaccine-preventable viruses continue to re-emerge globally, scalable multi-pathogen approaches are needed to generate actionable surveillance data from a single wastewater sample. To address this gap, we applied hybrid-capture viral probe panels (VSP) with whole-genome sequencing to concurrently detect multiple viral pathogens and characterize viral diversity, abundance, and temporal dynamics in communities across Alberta.

Nucleic acids were extracted from 100 mL 24h-composite wastewater samples collected weekly from 12 community wastewater treatment facilities in Alberta between December 2023 and May 2025 (n= 809). Libraries were prepared and sequenced using the Illumina RNA Prep with Enrichment (L) Tagmentation Kit, VSP v2 probes targeting 200 viruses, and the Illumina MiniSeq System. Viral detection, genome coverage, and abundance were analyzed using EsViritu v1.1.1, where Reads Per Kilobase per Million Filtered reads (RPKMF) denotes viral abundance.

Across samples, 666 unique viral references, including genotypes, sub-genotypes, and variants, were detected and consolidated into 56 biologically grouped virus categories. High-genome-coverage viruses (>90% covered bases) included polyomaviruses, enteric viruses, and respiratory coronaviruses, with abundance peaks reaching a maximum grouped RPKMF of 116,766.12. The wastewater virome was dominated by highly prevalent enteric viruses, with positivity ranging from 66.6% to 99.9%, including adenovirus (808/809, 99.9%), astrovirus (806/809, 99.6%), norovirus genogroup II (803/809, 99.3%), norovirus genogroup I (791/809, 97.8%), sapovirus (790/809, 97.7%) and rotavirus A (539/809, 66.6%). Strain diversity within these groups was also notable, with multiple astrovirus, norovirus, sapovirus, and rotavirus references detected across the study period. Beyond predominant enteric viruses, polyomavirus (806/809, 99.6%), coxsackievirus (757/809, 93.6%), rhinovirus (735/809, 90.9%), coronavirus 229E (792/809, 97.9%), and SARS-CoV-2 (787/809, 97.3%) were also highly prevalent. Respiratory viruses showed greater temporal variability. Based on BLAST-refined subtype assignments, Influenza A virus (IAV) was detected in 189 samples, with H1N1 predominating (120/189, 63.5%) and peaking in January 2024 and February-April 2025, while H3N2 (106/189, 56.1%) was most prominent in January-March 2025. Enterovirus D68 was detected at 24.8% positivity rate and peaked during the fall months (September-October 2024). Site-level heterogeneity was evident, with Bonnybrook- Calgary (BB) and Edmonton (ED) showing the highest overall virus diversity per sample (22.45 and 21.95 virus groups/sample, respectively), while Banff (BA) and Jasper (JA) showed relatively stronger influenza enrichment. Notably, Measles was detectable during provincial surge periods in a small subset of samples, with genotype B3 identified in 5 samples and genotype D8 in 7 samples, including the strongest D8 signal at the Lethbridge (LE) site in May 2025.

These findings demonstrate that hybrid-capture wastewater genomics can extend wastewater surveillance beyond single-target monitoring and provide a practical, real-time approach for tracking and resolving spatiotemporal patterns of co-circulating and low-frequency re-emerging viruses across Alberta. This scalable strategy strengthens integrated public health surveillance, enhances early warning capacity at the community level, and supports more responsive outbreak preparedness.

Gen abstract type

Poster

Section Type

AEM

306 - Comparative analysis of two epidemic strains of *Pseudomonas aeruginosa* infecting persons with cystic fibrosis in Southern Alberta

Melika Moradi[1, 2], Conrad Izydorczyk[3], Zhaohui (Shaelyn) Xu[3], Barbara J. Waddell[3], Iqramul Haq Haq[1], Mohammad Jokar[1], Christina Thornton[2, 3, 4], Michael G. Surette[3, 5], Harvey R. Rabin[3, 4], Diego Borin Nobrega[1], Michael D. Parkins[2, 3, 4]

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Background: Chronic airways infection with *Pseudomonas aeruginosa* is a major contributor to morbidity in persons with cystic fibrosis (pwCF). Some strains of this environmental organism are capable of pwCF-pwCF transmission. The Prairie Epidemic Strain (PES) was previously identified as a dominant clone in Western Canada, while the Liverpool Epidemic Strain (LES) is a well-recognized epidemic lineage that is more broadly disseminated. We investigated the long-term population structure, resistance phenotypes, and resistance gene profiles of serial isolates of these two epidemic clones to understand their pathobiology.

Method: Serial yearly isolates of *P. aeruginosa* recovered from 363 pwCF and non-CF attending the Southern Alberta Adult CF Clinic between 1986 and 2020 were assessed. Isolates underwent Illumina whole-genome sequencing. Reads were quality-checked with FastQC, trimmed using Trimmomatic, assembled with Unicycler, and annotated with Bakta. Sequence types were assigned in silico using PubMLST. Susceptibility to tobramycin, ceftazidime, ciprofloxacin, and meropenem was determined by Kirby-Bauer disc diffusion and interpreted according to CLSI guidelines. Multi-drug resistance (MDR) was defined as nonsusceptibility to ≥2 antimicrobial classes. Antimicrobial resistance genes (ARGs) were identified using the CARD database.

Results: A total of 1,652 *P. aeruginosa* isolates were recovered from 363 patients. MLST identified 160 distinct sequence types, although the collection was largely driven by a small number of dominant lineages, most notably PES (ST192; 406 isolates, 61 patients). PES was the most prevalent lineage and the only major clone detected consistently across all decades, supporting its long-term persistence in this population. Clone C (ST17), a well-recognized environmental lineage, represented 134 isolates, infecting 28 patients. LES (ST146 & ST683) was common within the 1980s but declined in prevalence in later years. 175 (10.59%) isolates were classified as MDR. Resistance was highest for tobramycin (16.95%), followed by ciprofloxacin (13.72%), ceftazidime (9.32%), and meropenem (6.21%). ST146 showed the highest phenotypic resistance across the four antibiotics, especially to tobramycin (72.7%), while ST192 displayed an intermediate but consistently elevated resistance profile. ARG presence/absence analysis showed a broad, shared core resistome across ST146, ST192, and ST683 compared with less-resistant, non-epidemic strains. ST683 carried the largest ARG repertoire, particularly aminoglycoside-modifying enzymes, whereas ST146 and ST192 showed stronger beta-lactam-associated signatures, including multiple PDC variants. AST patterns were broadly consistent with these genomic trends. A notable discordance between phenotypic resistance and ARG presence reflected adaptive mechanisms in chronically infecting strains as opposed to gene gain/loss.

Conclusion: *P. aeruginosa* across Southern Alberta was dominated by long-persisting epidemic lineages, particularly PES and LES. These strains showed distinct resistance profiles, with ST146 exhibiting the greatest phenotypic resistance burden. Although epidemic lineages shared a broad core genome, ARG differences explained AST patterns only partially, indicating that additional mechanisms such as point mutations and regulatory adaptation likely contribute to resistance. Future work integrating accessory genome and mutation-based analyses with isolate-level AST may better define the genomic basis of resistance.

Gen abstract type

Poster

Section Type

AEM

307 - Exploring Phage Therapy as a Precision Alternative for Urinary Tract Infection Prevention and Treatment Using an In Vitro Gut Model

Rachel Regier[1, 2, 3], Kait Al[1, 2], Scott Dos Santos[1, 2], Samantha Tapping[1, 2], Donna Goldhawk[1, 3, 4], Jeremy Burton[1, 2, 5]

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Urinary tract infections (UTIs) affect ~50% of females and frequently recur, causing substantial clinical and economic burden. Standard broad-spectrum antibiotic treatment can disrupt the gut microbiome and promote antimicrobial resistance. Phages, viruses that selectively kill bacteria, are an emerging therapeutic alternative. Uropathogenic *Escherichia coli* (UPEC), the leading cause of UTIs, can reside in the colon prior to infection, making this reservoir a target for preventative intervention. We aimed to investigate the efficacy of four commercially available *E.* coli-specific phages to reduce colonic *E. coli* while preserving microbiota diversity using an in vitro colonic model.

Each candidate phage was screened against 29 pure *E. coli* strains, including 14 UPEC isolates, using plaque assays. Next, a high-throughput in vitro colonic microbiota model (termed the "MiPro" model) was inoculated with optimized colonic media, *E. coli* UTI89 (UPEC strain lysed by T4, LL5 and LL12 phages in the plaque assay screen) and stool from three healthy human donors. Phage cocktail containing T4, LH01, LL5, and LL12 phage, as well as each phage tested individually (10^7 plaque forming units (PFU)/mL) were added and infectivity assessed at 24 and 48 hours. Total genomic DNA was extracted from stool slurries and microbiota composition and diversity was evaluated by V4-16S rRNA gene amplicon sequencing on an Illumina MiSeq. From this data, the relative abundance of live *E. coli* versus all other bacteria was analyzed using a two-way ANOVA with Tukey's post-hoc test.

Plaque assays indicated that each phage individually targeted different host strains within the *E. coli* species, however, over 90% of the tested UPEC strains were targeted by at least one of the four phages in pure culture. In vitro culture of stool slurries demonstrated that titers of individual infectious phages were highly donor-dependent, with some remaining stable while others increased by up to two logs or decreased by up to four logs. In contrast, the phage cocktail titer remained relatively stable across donors. In all three donors, amplicon sequencing showed a significant reduction in the relative abundance of *E. coli* after treatment with LL12 phage as well as the phage cocktail when compared to untreated controls at 24h (P <0.01) and 48 h (P <0.001). Microbial diversity (assessed using Shannon-Weaver diversity index) remained unchanged after phage treatment (P > 0.11).

These findings demonstrate selective depletion of *E. coli* in vitro without disruption of overall microbiota diversity. Targeting the gut reservoir of UPEC with phages may offer a strategy for UTI prevention while reducing reliance on preventative and therapeutic antibiotics.

Gen abstract type Poster

Section Type AEM

308 - Computational prediction of antibiotic resistance genes from environmental to human microbiomes

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Antimicrobial resistance (AMR) is a critical global public health issue projected to become a leading cause of death globally by mid-century. Antibiotic resistance genes (ARGs) are key determinants of AMR and form an ancient and intrinsic component of microbial ecosystems. Environmental bacteria vastly outnumber human-associated microorganisms, yet remain undercharacterized, and are widely recognized as major reservoirs of novel ARGs, a subset of which may be mobilized into clinically relevant pathogens. Identifying novel ARGs before their dissemination to human microbiomes and pathogens holds great potential clinical impact by informing surveillance, policy, and antibiotic development. Current computational approaches for detecting novel ARGs are constrained by a trade-off between maximizing the positive predictive value of experimentally validated predictions through reliance on high amino acid sequence similarity to known ARGs, and the challenge of accurately capturing the much larger pool of sequence-divergent novel ARGs.

We present ARG-PASS (ARG-PAirwise Sequence vs Structure), a novel computational tool designed to detect clinically relevant ARGs independently of sequence homology to known ARGs. Instead, ARG-PASS leverages structural similarity in functionally important protein regions by analyzing the relationship between pairwise sequence identity (seqID) and template modeling (TM)-score distributions. A one class support vector machine is trained to learn the decision boundary (DB) surrounding each seqID vs TM-score distribution, and if any points from the query protein structure fall within the DB of the relevant subject distribution, it is classed as functional. We aimed to validate ARG-PASS by expressing functional predictions in standardised expression systems for antimicrobial susceptibility testing against clinical breakpoints.

Applying ARG-PASS to an Indian lake metagenome and six Human Microbiome Project reference strains, we experimentally confirmed a diverse panel of novel ARGs including dihydrofolate reductases, beta-lactamases (class A, B3, C, and D), an aminoglycoside phosphotransferase, a penicillin-binding protein, and a *phnP* gene (metallo-beta-lactamase (MBL)-fold) sharing only 17.3% identity to known MBLs, providing a proof of concept of the tool's capacity to identify divergent resistance determinants. All predictions had activity above negative controls upon expression in *Escherichia coli* of the Minimal Antibiotic Resistance Platform, and 80% conferred clinically relevant minimum inhibitory concentrations against their respective antibiotics, with the remainder representing candidate "pre-resistance" genes. We suggest “pre-resistance” genes may preferentially evolve into clinically relevant resistance determinants.

Finally, given class B1 MBLs’ clinical relevance, we also used ARG-PASS to screen for them across environmental and human microbiomes from the MGnify database, and identified ~900 diverse potential candidate class B1 MBLs, a subset of which are planned to be experimentally verified. Future work will also leverage ARG-PASS to identify potential transmission routes of novel ARGs from environmental microbiomes which have high potential to reach human microbiomes.

ARG-PASS represents a precise, simple, and computationally efficient method of detecting previously uncharacterized ARGs from environmental and human microbiomes, particularly before they transfer to human pathogens.

Gen abstract type Poster

Section Type AEM

309 - Metagenomic Characterization of Influenza A Genotypes in Alberta Wastewater, 2023-2025

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Background: Influenza A (IAV) causes substantial morbidity and mortality worldwide through annual antigenic drift and occasional antigenic shift that can lead to global pandemics. Identifying circulating sub-genotypes and tracking viral evolution provide critical information for public health response and vaccination campaign. Wastewater-based surveillance (WBS) is a complementary tool for epidemiological assessment of community IAV activity. In this study, comprehensive whole genome sequencing of wastewater samples was conducted to characterize IAV diversity and examine temporal and geospatial trends across Alberta.

Material and Methods: Wastewater samples (n=801) collected weekly from 12 Alberta municipal wastewater treatment plants (WWTP) from Dec 2023 to Apr 2025 were included in this study. Sequencing and library-prep utilized Illumina Miniseq with Illumina® RNA Prep and enrichment by Tagmentation (L) kit. Primary bioinformatic analysis was performed using the EsViritu (v1.1.1) pipeline to identify and characterize viral pathogens, including IAV. IAV subtypes were further determined using BLAST-based sequence comparison. Clade and lineage assignments were conducted using the Nextclade (v3.21.1) platform. For phylogenetic analysis, a de novo evolutionary tree was constructed using IQ-TREE, with reference sequences obtained from the GISAID database.

Results: IAV was detected in 189 (24.1%) of the 785 samples that were successfully sequenced (785/801; 98%). H1N1 was found to be the most prevalent subtype (63.5%; 120/189), followed by H3N2 (56.1%; 106/189). H1N1 and H3N2 were co-presented in 21.1% of samples (40/189). All eight viral genome segments were observed collectively across samples; however, each individual sample generally contained only a subset of these segments. For each segment, most positive sequences range from 150 to 300 base pairs in length. There were significant differences in the total virus detection abundance in different sewer sheds. High levels of H3N2 were seen in Banff, Lethbridge and Jasper and of H1N1 in Banff, Bonnybrook and Grandie Prairie. Temporal trends showed that H1N1 and H3N2 was the predominant subtype alternatively over time. Highest influenza activity was identified in the winter months (from November to March) with peaked levels observed for both H1N1 and H3N2. Seventeen H1N1-positive and 25 H3N2-positive samples yielded hemagglutinin (HA) sequences. Sequence alignment revealed genetic variability among the HA genes. The predominant H1N1 lineage was H1N1_6B.1A.5a.2a and H1N1_6B.1A.5a.2a.1, and H3N2_3C.2a1b.2a.2a.3a.1 was the dominant H3N2 lineage. These strain profiles are consistent with the strains circulating in North America from 2023-2025. Wastewater H1N1_HA lineages clustered with contemporaneous circulating sequences from the same geographic region, representing community circulating strains. No distinct or divergent lineages unique to wastewater, as compared to Canada circulating strains, were observed.

Conclusions: WBS integrated with metagenomic sequencing provides near-real-time monitoring of circulating IAV strains prevalence for various communities in Alberta, revealing emerging variants of concern and their genomic evolution.

Gen abstract type

Poster

Section Type

AEM

311 - (meta)Genomic Evidence of Low-Temperature LDPE Degradation Across Canada's Lakes

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Microplastics (MPs) are pervasive contaminants in freshwater ecosystems, yet the ecological drivers shaping microplastic-associated microbial communities remain poorly resolved, particularly in cold inland waters. While microplastic biofilms often contain taxa with putative plastic-degrading capacity, it remains unclear whether plastic itself acts as a selective carbon source or merely a passive surface for colonization. Here, we investigated the capacity of freshwater bacteria to degrade low-density polyethylene (LDPE) under environmentally relevant, low-temperature conditions and assessed their broader ecological distribution.

We isolated 23 bacterial strains from microplastics collected in a boreal lake and a glacier-fed river and evaluated their growth on LDPE as the sole carbon source in mineral media. Several isolates removed up to 25% of LDPE (w/w), formed robust biofilms, and induced surface deterioration of the polymer. Gas chromatography-mass spectrometry detected alkanes, oxidation products, and plastic additives, consistent with LDPE depolymerization and microbial metabolism. Complete genome sequencing using Nanopore technology revealed homologs of known hydrocarbon- and plastic-degradation genes, suggesting the presence of previously undescribed degradation pathways. Notably, we identify members of the genera Sanguibacter and Cryobacterium as plastic-degrading bacteria for the first time.

We quantified the abundance of these taxa across the LakePulse metagenomic dataset, comprising whole-genome shotgun metagenomes from 308 lakes spanning Canada's major ecozones. We found that plastic-degrading taxa were widespread but typically rare, with abundance influenced by land-use type. Together, these results suggest that plastic-degrading bacteria reside within the freshwater rare biosphere and that microplastic inputs act as a selective pressure shaping the MP biofilm community.

Gen abstract type

Poster

Section Type

AEM

319 - Toward Genetically Defined Clubroot Research: Optimizing Single-Spore Isolate Multiplication and Phenotyping of Plasmodiophora brassicas

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Plasmodiophora brassicae, the causal agent of clubroot, is an obligate biotrophic protist that infects canola and other Brassicaceae crops. Despite its agricultural importance, research on this pathogen is frequently conducted using heterogeneous field isolates composed of multiple genotypes or pathotypes. The use of genetically homogeneous single-spore isolates (SSIs) remains limited because SSI recovery is technically difficult, time-consuming, and historically inefficient. Although recent advances have improved SSI generation, newly recovered isolates often yield very small amounts of gall tissue, creating a major bottleneck for downstream experimentation. In addition, SSI characterization requires greenhouse-intensive pathotyping assays using differential host systems.

The objective of this study was to develop greenhouse methodologies that support genetically defined clubroot research by: (i) maximizing inoculum production from low-yield SSIs, and (ii) evaluating whether reduced container size can improve pathotyping efficiency without compromising disease assessment.

For inoculum multiplication experiment, the universally susceptible host ECD 05 (*Brassica rapa* var. *pekinensis*'Granaat') was inoculated with 10^4 or 10^6 resting spores per plant and grown in containers of approximately 300, 800, or 2200 mL. Plants received 20N:20P:20K fertilizer once or twice weekly. Six weeks after inoculation, disease severity, gall biomass, and resting spore density were measured. For phenotyping efficiency, five host genotypes from the Canadian Clubroot Differential set were grown in plug-cell trays representing standard to reduced-space systems: 72-cell (~65 mL per cell, standard), 128-cell (~28 mL per cell), and 144-cell (16 mL per cell). Plants were inoculated with pathotypes 3H or 6B, and disease severity was assessed six weeks after inoculation.

In the inoculum multiplication experiment, at the lower inoculum level, disease severity increased with larger container volume and more frequent fertilization. Gall biomass increased strongly with larger container size and twice-weekly fertilization at both inoculum levels, with maximum production obtained in 2200 mL pots fertilized twice weekly. Plants inoculated with 10^4 spores under optimized conditions produced gall biomass comparable to those receiving 10^6 spores. In the phenotyping study, disease responses varied among host genotypes and between pathotypes. Container size did not significantly affect disease severity for pathotype 3H, whereas pathotype 6B produced higher severity in larger cells for several hosts. Reduced container systems were therefore not universally suitable for pathotyping.

These findings address two critical barriers to SSI-based clubroot research. Optimized multiplication conditions reduce the number of propagation cycles needed to scale newly recovered isolates, minimizing the risk of virulence shifts during repeated multiplication. However, reducing container size below the current standard for pathotyping may compromise disease expression in some host-pathotype combinations. Collectively, these methods support broader use of genetically homogeneous isolates, improving reproducibility, studies of virulence evolution, and resistance screening in canola breeding programs.

Gen abstract type Poster

Section Type AEM

322 - Surveillance of Shiga Toxin-Producing *Escherichia coli* (STEC) in Calgary and Across Alberta using Wastewater

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[1]University of Calgary, [2]University of Alberta, [3]Alberta Precision Lab-Provincial Laboratory, [4]Alberta Health Services

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are responsible for significant human morbidity can cause severe food-borne illnesses. The September 2023 STEC outbreak in Calgary daycares was among Canada's largest, affecting 448 individuals, including 38 children and one adult who required hospitalization, all linked to facilities with a shared kitchen. Understanding the epidemiology of STEC in an unbiased way is an important step in mitigating its impact. We sought to leverage a provincial SARS-CoV-2 wastewater-based surveillance (WBS) program to investigate genomic targets for STEC and understand how these correlated with clinically identified disease.

Methods: Composite 24-hour wastewater samples were collected monthly from geographically and socioeconomically diverse communities (n=5) across Alberta at the level of wastewater treatment plants (WWTPs) from April 2022 to March 2024. During the Calgary daycare outbreak samples were collected weekly from August 2023 to November 2023 from municipal WWTPs, six urban neighbourhoods, and the Regional Pediatric Hospital (RPH-1). Additional monthly samples from RPH-1 and WWTPs were gathered between March 2023 and March 2024. Samples underwent pelleting and DNA extraction using the Qiagen DNeasy PowerSoil Pro kit. Extracts were evaluated for four potential genomic STEC targets: Shiga Toxin 1 (*stx1*), Shiga Toxin 2 (*stx2*), Intimin (*eae*), and LPS O antigen gene specific for O157 ($rfbE_{O157}$), by multiplex PCR. Each target was normalized to 16S rRNA gene abundance to account for the total bacterial load. Amplicons of *fliC* (H antigen), *stx1* and *stx2* were sequenced using Oxford Nanopore technology in selected samples to understand 'strain'-level diversity. STEC genomic targets were assessed using Spearman's rank correlation and differences were compared using the Mann-Whitney U-test.

Results: Wastewater-measured STEC gene targets displayed seasonal trends across Alberta communities, peaking in summer months with clear regional variation[BW1] [BW2] . STEC Target exhibited strong correlations across sites (*stx1* vs *stx2*, (r=0.802, p<0.0001); *stx2* vs $rfbE_{O157}$, (r=0.634, p<0.0001); *eae* vs $rfbE_{O157}$, (r = 0.551, p<0.0001);*stx2* vs. *eae*(r=0.542, p<0.0001). During Calgary's daycare outbreak, spikes in STEC gene targets occurred, with RPH-1 showing a pronounced median increase in $rfbE_{O157}$ and *stx2* in September 2023 compared to March-August and October-November 2023. Similarly, levels of $rfbE_{O157}$ and *stx2* doubled across Calgary WWTPs, with significant increases in STEC genomic targets observed in monitored neighbourhoods relative to the non-outbreak periods. Smaller sewershed catchments exhibited greater variance than municipal WWTPs. Amplicon sequencing confirmed the presence of O157:H7-associated *fliC* H7, *stx1a* O157, and *stx2a* O157 in samples positive for STEC targets during the daycare outbreak.

Conclusions: WBS enables non-invasive, population level-screening for infectious diseases including STEC. STEC gene targets in community wastewater aligned with established seasonal disease patterns (PMID 31652648). All four genomic targets of STEC demonstrated significant correlations across various sewersheds. During the daycare-associated outbreak, STEC gene targets increased across multiple spatial scales and samples tested positive for O157:H7-specific sequences through amplicon sequencing. Real-time WBS may therefore serve as a valuable supplementary approach monitoring and responding to large-scale enteric pathogen outbreaks, augmenting case-based surveillance.

Gen abstract type

Poster

Section Type

AEM

324 - Subinhibitory concentrations of antimicrobial peptides activate resistance mechanisms and virulence in Vibrio cholerae

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Polymyxins are antimicrobial peptides (AMPs) used in agriculture to treat and prevent bacterial infections, and as last-resort therapeutics against multidrug-resistant infections in humans. Due to their poor absorption, they can enter aquatic environments through runoff. Because sub-inhibitory concentrations of AMPs can act as signaling molecules for bacteria, we hypothesize that environmental AMP pollution may alter microbial community interactions, enhance the virulence and resistance of pathogenic bacteria, and promote their persistence in both environmental and clinical contexts.

The objective of this project is to determine the effects of sub-inhibitory concentrations of AMPs on survival determinants in Vibrio cholerae, a marine bacterium responsible for cholera, and to assess their role in bacterial persistence.

Using genomic, transcriptomic, and proteomic approaches, we show that V. cholerae, in response to sub-inhibitory concentrations of AMPs, produces large membrane vesicles that sequester AMPs and reduce their antimicrobial activity. We also identified a dedicated operon involved in AMP resistance that enables AMP sensing and activates multiple resistance mechanisms upon exposure. In addition, the Type VI secretion system, which plays a key role in interbacterial competition, colonization, and virulence, is enhanced under sub-inhibitory AMP conditions.

Taken together, rather than acting solely as antimicrobial agents, our findings demonstrate that AMP pollution can activate key survival mechanisms in V. cholerae.

Gen abstract type

Poster

Section Type

AEM

325 - Evolutionary and Phenotypic Dynamics of Salmonella enterica under Long-Term Low-Moisture Storage Conditions

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Introduction: Low water activity foods are generally considered microbiologically stable due to their inability to support microbial growth. However, *Salmonella enterica* can persist in these environments for extended periods under desiccation stress, maintaining viability and posing a latent food safety risk. While short-term stress responses are well characterized, the long-term genomic and phenotypic adaptations that facilitate persistence in low-moisture foods remain poorly understood.

Purpose: This study investigated the long-term persistence and adaptive responses of *Salmonella enterica* serovars Muenchen and Cubana during six months of storage in low-moisture food matrices, with a focus on identifying genomic variations and associated phenotypic changes.

Methods: Wheat, sunflower seeds, walnuts, and hazelnuts were inoculated with *Salmonella* (Muenchen and Cubana) at approximately 10^7 - 10^8 CFU/g and stored under ambient conditions (24^0C; 25-35% RH) for up to 6 months. At two-week intervals, samples were enumerated, and isolates were selected for whole-genome sequencing (WGS) on an Illumina MiSeq, with parental strains sequenced using both Illumina and Oxford Nanopore technologies for hybrid assembly. High-confidence non-synonymous SNPs were identified using the Snippy tool. Phenotypic profiling was conducted using Omnilog Phenotype Microarrays (PM09 plates), and metabolic activity was quantified using DuctApe to determine fitness gains and losses relative to parental strains. Genome-phenome integration was performed using KEGG-based functional annotation.

Results: Limited genomic variation was observed in *S*. Muenchen, with a small number of non-synonymous mutations primarily identified in walnut and wheat matrices, including genes associated with DNA repair (sbcC), metabolism (bioD1_2), respiration (torD_1), and stress adaptation (*dpaL*). In contrast *S*. Cubana had a dominant recurring frameshift mutation in the gmd gene across all matrices and timepoints, along with additional mutations in genes linked to osmotic stress (*proW*), redox regulation (*arcB*), metabolism (*nadB*, *aroC*), DNA repair (*mutY*, *recQ*), and membrane function (*fabH*). Phenotype profiling revealed that both serovars were characterized by reduced fitness under osmotic and chemical conditions, particularly in urea (3-5%), sodium nitrite (60-100mM), and ethylene glycol. However, condition-specific gains were observed in osmoprotectant-associated environments, including NaCl supplemented with trehalose.

Significance: *Salmonella* demonstrated the capacity to persist in low-moisture food environments with minimal but functionally relevant genomic adaptation over six months. While phenotypic responses were largely characterized by reduced metabolic activity under stress conditions, selective advantages in osmoprotectant environments suggest targeted adaptive responses. The integration of genomic and phenotypic data provides new information on the mechanisms underlying persistence in desiccated environments, highlighting the potential for subtle evolutionary changes to influence survival and risk in low-moisture food systems.

Gen abstract type Poster

Section Type AEM

326 - Composite severity scoring and image-based phenotyping reveal interaction-driven disease expression in the Prairie Root-Rot Complex

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The Prairie Root-Rot Complex (PRRC) is a disease caused by a consortium of soil-borne pathogens that collectively limit pea (Pisum sativum) production in the Canadian Prairies. Current disease assessment methods use pathogen-specific rating scales that inadequately capture the combined symptom expression arising from mixed infections. To provide a standardized approach for quantifying disease expression under multi-species infections, we developed a unified composite severity scale (0-5). Field microplot observations across three Saskatchewan sites showed substantial variation, with disease phenotypes often dominated by Aphanomyces and Rhizoctonia at Regina, and by Aphanomyces and Fusarium at Swift Current, despite molecular confirmation that all three taxa were present at all locations. A total of 247 field-derived pathogen isolates were recovered and identified through multi-locus phylogenetics as Fusarium spp. (20.24%; Fusarium avenaceum, F. equiseti, F. graminearum, F. redolens, F. solani), Rhizoctonia solani AG-4 HGII (8.10%), and Binucleate Rhizoctonia AG-K (2.43%). To assess the individual and combined contribution of these taxa to disease development, we performed structured single, dual, and triple inoculations under controlled conditions. To ensure consistent scoring and enable computational validation of the composite scale, all roots were imaged under standardized conditions, generating a curated dataset. This dataset is being used to train machine-learning models for independent phenotypic scoring and validation of the composite severity scale. Solitary infections established baseline aggressiveness with A. euteiches and F. avenaceum producing the highest composite severity scores while F. solani and R. solani AG-4 HGII generated moderate scores, and Binucleate Rhizoctonia AG-K induced only mild symptoms. When assembled into mixed communities, combinations containing A. euteiches with either F. solani or R. solani consistently shifted into the upper composite severity classes (4-5), whereas assemblages lacking A. euteiches remained in moderate ranges. The composite severity scale captures field-realistic disease expression in the PRRC and provides a standardized approach for quantifying disease severity caused by multiple pathogens in pea.

Gen abstract type

Poster

Section Type

AEM

330 - Targeting and specialization of highly similar interbacterial weapons

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Across communities from diverse environments, bacteria interact with each other, often competing for limited space and resources. Bacteria even directly attack and kill their rivals using a range of molecular weapon systems. Despite the wide array of different known weapons, bacterial genomes have been observed to encode multiple similar weapons. As they appear to perform the same function of killing competitors, we investigated whether there are additional benefits of carrying multiple similar weapons. Using genomics to compare strains of the heavily-armed opportunistic pathogen, *Pseudomonas aeruginosa,* we found that multiple similar bacteriocins (S-pyocins) are commonly encoded in the genomes of many strains. By encoding more pyocins, these strains increase the number of possible competitor strains that can be effectively attacked. However, we also found that encoding a single uncommon S-pyocin is just as effective as several, meaning this use is unlikely to provide the selective pressure for encoding multiple pyocins. We thus hypothesized that the simultaneous use of multiple pyocins provides a combinatorial benefit. Testing this hypothesis using single and double deletion mutants of the type strain PAO1 revealed that for most conditions and pyocin pairs, there was no benefit to using of multiple pyocins compared to a single pyocin. As S pyocins are imported via siderophore receptors, we then tested whether environmental iron availability influenced their effectiveness and found this is indeed the case: pyocin S5 is much more effective than S2 when iron was limited, and vice-versa when iron was restricted. We then generalized our findings by demonstrating that a completely distinct weapon, contact-dependent inhibition, also shows environmental condition-dependent utility. From this work, we conclude that bacteria encode weapons that seem very similar, but they are actually specialized for fighting in different environmental circumstances.

Gen abstract type

Poster

Section Type

AEM

342 - Efficacy of biological products against Clavibacter michiganensis on tomato plants under greenhouse conditions in Québec

Jose Alejandro Barboza Del Toro[1, 2, 3], Edel Perez-Lopez[1, 2, 3], Anne-Sophie Brochu[1, 2, 3], Elisa Ines Fantino[1, 2, 3], Justine Brillet[1, 3]

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Tomato bacterial canker is a devastating seed-borne disease caused by *Clavibacter michiganensis* (Cm), a non-spore-forming Gram-positive bacterium responsible for significant yield losses worldwide. In Quebec, Cm remains one of the most difficult greenhouse diseases to control, due to the lack of genetic resistance and the limited effectiveness of current management strategies. In this project, we characterized a collection of ten Cm isolates using whole-genome sequencing, allowing us to describe their genetic diversity as well as the composition of their plasmids. In parallel, we evaluated the response of tomato plants to these different isolates, highlighting variability in host-pathogen interactions. We also analyzed Bacillus-based biocontrol agents, whose genomes were sequenced to predict metabolites potentially involved in antagonistic activity against Cm. The efficacy of these strains was assessed under controlled conditions, and their interaction with the plant was studied. Finally, transcriptomic analyses were used to compare the tomato response to infection by Cm alone and in the presence of Bacillus consortia, revealing changes in defense pathways and responses associated with disease suppression. Together, these results provide an integrated view of the interactions among Cm, biocontrol agents, and the host plant, and pave the way for the development of more sustainable management strategies for tomato bacterial canker.

Gen abstract type

Poster

Section Type

AEM

344 - Comparative analysis of biofilm communities present within an organic greenhouse irrigation system over a one-year period

Morgan Nolan[1], Marc Habash[1]

[1]University of Guelph

This research is focused on the management of biofilms in an organic greenhouse irrigation system. Biofilms are an aggregation of microbial organisms that form on a biotic or abiotic surface and are held together by their secretion of various macromolecules and compounds. Biofilms are a significant and ongoing issue in greenhouse irrigation systems that result in crop losses, impact water sustainability, and health and safety of workers and consumers of greenhouse products. This research is studying biofouling within an organic greenhouse irrigation system with the goal of identifying strong biofilm forming bacteria and developing a specified treatment capable of targeting those bacteria. This research focuses on the characterization of the bacterial diversity within the greenhouse irrigation system. The data presented is a comparative analysis of the data collected from three separate sampling events, held in Winter 2025, Summer 2025, and Spring 2026. Biofilm samples from the nozzles and filters were collected and bulk water samples within their irrigation system during each sampling event. DNA was extracted from each of the samples and analyzed by 16S amplicon sequencing to assess bacterial diversity. Long-read and short-read sequencing were performed for both 2025 sampling dates, and long-read sequencing will be used for the 2026 sampling. The short-read sequencing from the 2025 sampling events concluded that the genera Pseudomonas, Bacillus, and Janthinobacterium were among the top three most abundant overall within the irrigation system. Testing on bulk water and sampled biofilms exhibit differences in microbial diversity and abundance, but Janthinobacterium and Bacillus were consistently among the top three most abundant genera. We expect the long-read sequencing of the 2026 sampling event to yield similar genera to those of 2025, with a higher resolution with identification to the species level due to the increased length of 16S gene fragments. There is no literature currently existing that has characterized biofilm communities in an irrigation system down to the genera or species level. This research contributes to our understanding of bacterial diversity within greenhouse irrigation system biofilms to help with the management of chronic biofouling. These data will be used to design and test biological treatments for biofilm management from a selection of essential oils, enzymes, or a combination of both as a part of a 2-year study.

Gen abstract type

Poster

Section Type

AEM

347 - Functional Metagenomic Discovery of Kanamycin Resistance Determinants from Wastewater Metagenomes

Sherry Azeez[1], Trevor Charles[1], Andrew Doxey[1]

[1]University of Waterloo

Municipal wastewater represents a critical intersection between environmental, microbial, and human-associated ecosystems and serves as an important reservoir for antimicrobial resistance (AMR). While wastewater-based surveillance has emerged as a powerful tool for monitoring resistance trends, most current approaches rely on sequence homology to known reference databases, limiting their ability to detect novel or highly divergent resistance determinants. The objective of this study is to identify phenotypically active kanamycin resistance mechanisms in wastewater that evade conventional sequence-based detection. To address this gap, a cosmid-based functional metagenomic library constructed from influent municipal wastewater was screened using a multi-host workflow to reduce host-specific expression bias. Environmental DNA was initially selected for kanamycin resistance in *Pseudomonas putida*, followed by plasmid recovery and validation in *Escherichia coli*. Resistant clones were prioritized for long-read sequencing, assembly, and bioinformatic analysis, including interrogation against the Comprehensive Antibiotic Resistance Database and open reading frame prediction. Several kanamycin-resistant clones were identified, including a focal clone that reproducibly conferred resistance across hosts despite lacking any annotated aminoglycoside resistance genes. Ongoing subcloning and functional validation aim to resolve the genetic basis of this resistance phenotype. These findings highlight the limitations of homology-based AMR surveillance and reinforce the value of integrating functional metagenomics with wastewater monitoring. By revealing unannotated resistance determinants at the human-environment intersection, this work advances a One Health framework for early AMR detection and supports proactive public health strategies aimed at mitigating emerging resistance threats.

Gen abstract type

Poster

Section Type

AEM

348 - Optimizing Oat Milk Kefir and Dairy Starter Cultures via Supplementation and Atmospheric Control

Evelyn Ratke[1], Joseph Sebastian[1], Benjamin C.T. Bourrie[1,2], Benjamin P. Willing[1]

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Kefir is a fermented beverage produced through complex microbial interactions that confer diverse health-promoting properties. Variability in kefir grain type, composition and dependence on initial substrates limit commercial standardization and present challenges to the application in plant-based alternatives. Previous studies in our lab showed that kefir produced using a mixed microbial consortium isolated from traditional kefir afforded greater health benefits than commercially available kefir. The present study evaluates the feasibility of producing oat milk kefir using the same consortium for traditional milk kefir by optimizing fermentation through substrate supplementation, and evaluating the effects of atmospheric control on starter culture production.

To this effect, oat milk fermentations were conducted with or without brown sugar or oat malt supplementation to enhance fermentable substrate availability using the microbial consortium. Fermentation performance was monitored through quantifying temporal pH, determination of microbial composition using differential plating, and quantification of organic acids produced via high-performance liquid chromatography (HPLC). In parallel, the effect of carbon dioxide on starter culture performance was assessed by comparing dairy kefir fermentations with starter cultures grown with or without 5% CO_2 atmosphere. The temporal pH and microbial composition were determined along with HPLC quantification of metabolites.

Overall, this study aimed to determine the suitability of oat milk as a kefir substrate and assess whether controlled environmental conditions can enhance starter culture performance. Carbon dioxide had no significant effect on differential yeast colony formation unit (CFU) counts, though had an observable effect on some bacteria. *Lactococcus lactis* grew better under 5% CO_2 conditions, with mean CFU counts at the end of the 24 hour fermentation being $3.9x10^{11}$ CFU/ml, as compared to non-CO_2 enriched conditions ($3.4x10^{9}$ CFU/ml). Conversely, *Lactobacillus kefiri* was found to grow better without CO_2 supplementation ($5x10^{8}$ CFU/ml) than under 5% CO_2 ($4.1x10^{7}$ CFU/ml). These indicate that starter culture production may be controlled with the manipulation of the atmospheric environment under which they grow.

Surprisingly, oat milk fermentation achieved a food safe pH (i.e.<4.5) more quickly than milk fermentation. After 15 hours, oat kefir had achieved a pH of 3.96, whereas milk kefir was at pH 4.73. Supplementation with brown sugar or oat malt did not further accelerate fermentation, achieving a pH of 3.99 and 4.00 respectively at 15 hours. It can be determined that oat milk may be a suitable substrate for plant-based kefir production, however, fermentation parameters may need to be adjusted to control the acidity and metabolites produced.

Gen abstract type

Poster

Section Type

AEM

350 - Wastewater-based surveillance of Streptococcus pyogenes and Streptococcus pneumoniae: A targeted molecular approach for strain-level identification

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Introduction: Invasive bacterial infections caused by *Streptococcus pyogenes* (Group A Streptococcus, GAS) and *Streptococcus pneumoniae* (pneumococcus) represent a growing population health burden, with rising incidence and disproportionate impacts among vulnerable groups. Whereas GAS is most commonly responsible for pharyngeal and skin and soft tissue infections - including necrotizing fasciitis and toxic shock syndrome, pneumococcus is more commonly associated with pneumonia and meningitis. In Canada, the burden of these pathogens is increasing. Invasive GAS incidence doubled from 2.7 to 5.6 cases per 100,000 between 2001 and 2021, while invasive pneumococcus incidence rose from 5.6 to 10.2 cases per 100,000 between 2021 and 2022. Both species exhibit substantial strain-type diversity. Furthermore, specific strain types are associated with greater risk of incidence and worse clinical outcomes, underscoring the need to track specific lineages. Over 240 *emm* and 100 capsular polysaccharide (*cps*) types have been described to date, reflecting the substantial diversity of these pathogens. Because colonization precedes invasive disease, monitoring community carriage is essential for outbreak prediction and infection mitigation. However, conventional surveillance remains reactive and biased toward severely symptomatic individuals, creating gaps in our understanding of circulating strain diversity at the population level. Wastewater-based surveillance (WBS) offers a passive, inclusive, cost-effective, near-real-time, community-wide approach for early detection of high-risk lineages. This project aims to develop and validate a targeted molecular approach integrating quantitative PCR (qPCR) and amplicon sequencing into a WBS workflow to identify circulating strains across a range of population scales (e.g., homeless shelters to entire cities).

Methods: To quantify pathogen abundance, a duplex qPCR assay was developed using two genetic targets per organism: *spy1258* and *speB* for GAS, and *lytA* and SP2020 (locus tag) for pneumococcus. This dual-target approach reduces false positives from closely related species and improves detection confidence. Initial primer and probe functionality was confirmed using synthetic gene targets (gBlocks), with full assay validation using mock communities of known strain-types spiked into phosphate-buffered saline and wastewater to assess sensitivity, efficiency, limits of detection, and sequencing bias.

Results: Initial metagenomic analysis confirmed that pneumococcal and GAS reads accounted for less than 0.01% of total bacterial reads within 105 wastewater metagenomes (median ≈33 million/reads per sample). This very low burden of target relative to background genomic material suggests that untargeted sequencing approaches are insufficient, validating our targeted strategy. For strain-level resolution, isolates and wastewater samples were extracted using the QIAGEN DNeasy PowerSoil Pro Kit, followed by downstream PCR amplification of the *emm* and *cpsB* genes. The *emm* and *cpsB* targets enable interspecies discrimination while preserving sufficient intraspecies variability for strain differentiation and are supported by established reference databases. Preliminary results demonstrate successful amplification of the *cpsB* region from wastewater, with Nanopore sequencing yielding high-quality reads across all samples (mean Q score > Q34).

Conclusion: This integrated molecular-WBS approach provides a scalable, proactive early-warning system capable of tracking high-risk lineages across varying geographic scales. This enables population-level tracking of strain dynamics that can be overlayed with clinical data streams to inform timely public health responses and intervention strategies.

Gen abstract type Poster **Section Type** AEM

355 - Fed-Batch Cultivation of *Methylomicrobium album* BG8 on Methanol for C1 Valorization

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Background: Methanotrophic bacteria are promising platforms for converting single-carbon (C1) compounds into biomass and value-added products, including bioplastics, fuels, and organic acids. While methane is an energy-rich and abundant feedstock, its use as a feedstock in bioreactors is limited by gas-liquid mass transfer constraints and process complexity. Methanol, which is a common industrial byproduct, provides a practical liquid alternative that enables more efficient and controllable cultivation. Among methanotrophs, *Methylomicrobium album* BG8 has emerged as a robust model organism capable of efficient growth on methanol. However, its cultivation has been largely limited to shake flask systems, and its performance under controlled bioreactor conditions remains insufficiently characterized for scalable and productive processes.

Objective: This study investigates the cultivation of *M. album* BG8 using methanol as the sole carbon source in controlled fed-batch bioreactor operation as a strategy toward scalable C1-based bioprocessing.

Methods: Cultivations were performed in stirred-tank bioreactors under aerobic conditions at 30°C, with controlled pH and dissolved oxygen. Batch and fed-batch strategies were evaluated to assess growth behavior and process performance. During fed-batch operation, methanol was supplied as the limiting substrate. Biomass formation was monitored over time by measuring optical density at 540 nm (OD540).

Results: *M. album* BG8 grew under the tested conditions using methanol as substrate. In batch mode, biomass reached OD540 of 2 after 36 h, after which the process was transitioned to fed-batch operation. Biomass accumulation was sustained up to OD540 of 40 after 120 h, corresponding to 7.4 g/L on a cell-dry weight basis, resulting in a 20-fold increase in biomass content compared to batch cultivation. Residual methanol concentrations remained below the detection limit throughout fed-batch operation, indicating efficient substrate usage and carbon-limited conditions.

Conclusion: This work represents a step toward controlled bioreactor cultivation of *M. album* BG8 using methanol as a C1 feedstock at scale and provides a basis for future production from a metabolic engineering platform. In particular, this approach may enable redirection of carbon flux toward the production of higher-value compounds, such as precursors for bioplastics (e.g., lactic acid for PLA), supporting the development of sustainable methanol-based bioprocesses.

Gen abstract type

Poster

Section Type

AEM

360 - The impact of Enterobacteriaceae on spontaneous beer fermentation

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Spontaneous beers are more complex in their sensory attributes than conventional beers due to the metabolic activities of autochthonous yeast and bacteria that inoculate the cooling wort from the atmosphere. This results in a complex microbial community structure and an observed microbial succession during fermentation, starting with an enterobacterial phase that is unique to this style of beer. However, the impact of the *Enterobacteriaceae* family on the fermentation and the activities of other microbes involved in later stages of active fermentation, acidification and maturation remains underreported. Therefore, this study aims to elucidate the effect of enterobacteria during spontaneous beer fermentation, microbial community dynamics and flavour development. To this end, small scale fermentations using brewery wort from Blind Enthusiasm Brewing Company, specializing in spontaneous beers, were conducted at room temperature for 3 months. The fermentations were performed using a core community, comprising *Saccharomyces cerevisiae*, *Acetobacter cerevisiae*, *Levilactobacillus brevis* and *Brettanomyces bruxellensis*, in the presence and absence of enterobacteria, such as *Enterobacter kobei* and *Pantoea* sp.. The two enterobacteria were selected based on their differing ability to decarboxylate ferulic acid and subsequently produce 4-vinyl guaiacol during fermentation. Furthermore, to identify the ideal pitching regiment, experiments were conducted using either simultaneous pitching of all organisms or a sequential pitching strategy, where the core community was pitched following a 1-week enterobacterial pre-fermentation. The preliminary results of the ongoing study have shown a considerable shift in the fermentation profile based on the pitching regiment as well as perceptible shifts in the flavour of the beer. Additionally, culture-based evaluation of microbial community structure reveals that the *E. kobei* pre-fermentation resulted in a 1-log reduction in the growth of *S. cerevisiae* after a week of fermentation following pitching of the core community. A similar reduction in *L. brevis* growth was also observed for these *E. kobei* pre-fermentations. However, this profound impact on microbial growth was not observed for the *Pantoea* sp. pre-fermentation. To better understand the impact of the presence of enterobacteria during fermentation, HPLC and two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGX-TOFMS) will be performed on temporal samples collected during the course of the fermentation. Thus, the results from these investigations will improve our understanding of the role of the enterobacterial phase in microbial community assembly and flavour production during spontaneous beer production.

Gen abstract type

Poster

Section Type

AEM

362 - Rapid emergence and clonal expansion of multidrug resistant *Citrobacter werkmanii* in food animal reservoirs

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Citrobacter werkmanii is an underrecognized member of the Enterobacterales with increasing relevance to antimicrobial resistance (AMR) emergence, yet its population structure and resistance burden in food-animal-associated settings remain poorly defined. Here, we investigated the genomic epidemiology of C. werkmanii recovered from food animals and related sources and assessed evidence of recent lineage expansion within a One Health framework.

Forty-eight *C. werkmanii* isolates were recovered from food animals, predominantly chicken-associated, but also including bovine, environmental water, and captive wild bird sources, between 2016 and 2021 in Ilorin, Nigeria. Whole-genome sequencing was performed on the Illumina NextSeq 1000 platform, and the resulting genomic data served as the basis for phylogenomics and gene-content analysis with standard bioinformatic pipelines. Multidrug resistance was defined as the presence of genes conferring resistance to at least three different antimicrobial classes.

Substantial temporal phylogenomic structure was observed. Eight clusters defined at ≤20 core SNPs accounted for 77% (37/48) of the genomes, including two dominant clusters of 10 isolates each that were indistinguishable by SNPs, indicating high clonality within these lineages. Screening for acquired AMR determinants revealed β-lactam and quinolone resistance genes to be predominant in the collection, with *blaCMY/blaCMY-49* and *qnrB/qnrB18* forming a conserved resistance backbone. Additional frequently detected AMR genes included *tet(A), sul1, floR, qnrS1, aph(3')-Ia, dfrA17*, and *aadA5*. Overall, 35% (17/48) of isolates were multidrug resistant.

The principal MDR lineage comprised 21% (10/48) of the genomes and showed pairwise distances of 0-5 SNPs, with nine members of this cluster sharing an expanded accessory resistome that included *aadA5, aph(3')-Ia, floR, qnrS1, sul1, tet(A),* and *dfrA17*. Notably, biocide (*qacEΔ1*) and arsenic resistance determinants (*arsABCDR*) were also present. Another lineage containing 10 isolates was similarly clonal (0-16 SNPs) but retained a much narrower resistome (*blaCMY and qnrB*) with one isolate additionally carrying *dfrA14*, *qnrS1*, and *tet(A)*.

Comparison of these isolates with 86 global *C. werkmanii* genomes retrieved from public repositories showed that 23% (11/48) of the food-associated isolates in this collection were indistinguishable (0 SNPs) from publicly available genomes, indicating circulation of closely related lineages beyond the local setting.

Taken together, these findings support the coexistence of a broadly distributed cephalosporin- and quinolone-resistant *C. werkmanii* background and a more derived multidrug-resistant sublineage undergoing clonal expansion in food-animal reservoirs. These data further position this bacterium as a rapidly emerging One Health pathogen with potential implications for the wider ecology of multidrug resistance.

Gen abstract type Poster **Section Type** AEM

365 - Enzyme-Assisted debittering and purification of enzymes for the preparation of a bioactive-enriched kinnow beverage

Manmeet Chani[1], Neha Sharma[1], Shivani Sharma[1], Parampal Sahota[1]

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The commercial processing of kinnow fruits into beverages has been limited due to inherent bitterness, predominantly attributed due to the accumulation of limonoids and flavonoids. To address this issue while maintaining the sensory acceptance of the beverage, the present study employed the production of purified debittering enzymes, specifically limonin dehydrogenase, naringinase, and β-glucosidase isolated from *Clavispora lusitaniae*. The limonin dehydrogenase enzyme has been purified to 1.14- fold with 33.98% recovery, 29.25 IU activity and 0.525 IU/mg specific activity resulted in 86.43% degradation of limonin content in beverage by catalyzing oxidation of limonin -A-ring lactone to the non-bitter 17-dehydroxylimonoate. The debittering naringinase enzyme showed 24.8 IU activity ,0.442 IU/mg specific activity, purified to1.68 fold found to hydrolysed naringin by 57.93% into non-bitter bioactive compound naringenin. The flavour enhancing β-glucosidase with activity 14.68 IU, specific activity 0.344 IU/mg purified to 1.18-fold resulted in the increase in glucose content upto 4.25µg/ml in bioactive enriched kinnow beverage. Furthermore, using the debittered enzymes, bioactive enriched kinnow beverage has been developed with the bioprocess conditions as TSS 13°B, yeast inoculum @ 0.75%(v/v), incubation temperature 28±2°C and time 36 hrs. The physicochemical changes in bioactive enriched kinnow beverage after 25 days of storage at 4°C were recorded as TSS 13°B, acidity 0.313%, naringin 181.13 ppm, limonin 4.73 ppm, glucose 1.3µg/ml, total sugars 42.97 µg/ml, ascorbic acid 34.22 mg/ml. The maximum reduction in bitter component limonin 87.34%, naringin 58.41% whereas increase in glucose upto 4.38 µg/ml, acidity 30.13% and total sugar content 42.97 µg/ml has been analyzed. The nutraceuticals recorded in bioactive enriched kinnow beverage were flavonoids (mg/L)- gallic acid 0.075, caffeic acid 0.002 , rutin 0.001,ferulic acid 0.001 ; Organic acids (mg/L) - oxalic acid 0.243,tartaric acid 3.698, mallic acid 0.018, citric acid 0.432; Fat soluble vitamins (mg/L)- Vitamin A (Retinol) 0.060 , cholecalciferol (D3) 0.038 , α-tocopherol (Vitamin E) 0.001, Vitamin K 0.018 ; water soluble vitamins (mg/L)-thiamine 0.782, pyridoxine hydrochloride 0.040, pantothenic acid 0.002, riboflavin 0.001, biotin 0.011; Protein 6.42g/100g ,Carbohydrate 40.95g/100g.

Key words: Limonin dehydrogenase, Naringinase and β-glucosidase, *Clavispora lusitaniae*, Purification, Bioactive enriched kinnow beverage

Gen abstract type

Poster

Section Type

AEM

366 - Methanol-Based Lipid Production with *Methylorubrum extorquens* and *Auxenochlorella protothecoides*

Angie N Lizarazo-Roman[1], Bernardo A Souto[1], David C Bressler[1]

[1]University of Alberta

Microbial lipids are a promising renewable source for liquid biofuels, but it is still difficult to make this work at large scale because of process stability and conversion efficiency. Methanol is an interesting carbon source because it can be easily, it mixes well with water, and it can be fed in a controlled way in bioreactors. However, methanol is toxic for many microorganisms, and small tolerance windows can reduce growth and lipid production.

This project will develop a bioprocess approach for methanol-to-lipids using two microorganisms in parallel: the oleaginous microalga *Auxenochlorella (Chlorella) protothecoides* UTEX 256 and the methylotrophic bacterium *Methylorubrum extorquens* OSD20. *A. protothecoides* is selected because it is known for high lipid production, but it is not naturally adapted to grow on methanol, so methanol handling is a major challenge for this organism. *M. extorquens* naturally grows on methanol and is a good reference organism for methanol tolerance, but it does not behave like a typical TAG-accumulating organism, so the lipid outcomes need to be evaluated carefully.

First, screening experiments will be done in shake flasks to identify methanol tolerance thresholds and to test cultivation conditions (pH, temperature and nutrient availability) that support growth and lipid production. Then, the most promising conditions will be transferred to lab-scale bioreactors operated in batch and fed-batch modes to evaluate stability, reproducibility, and basic scale-up feasibility under controlled pH, temperature, and feeding. Random UV mutagenesis and adaptive laboratory evolution (ALE) will be applied to improve methanol tolerance and lipid productivity under methanol exposure. Lipid content and fatty acid profiles will be measured using lipid extraction and chromatography-based analysis.

Finally, lipid-rich biomass from selected conditions will be evaluated using two-step thermal conversion to measure hydrocarbon yields and product profiles, as a way to connect the upstream bioprocess to fuel relevance. Overall, the project will provide a clear dataset linking methanol handling strategies with microbial performance and lipid outputs, supporting decisions on whether methanol-to-lipids bioprocessing can be a realistic pathway for biofuel applications.

Gen abstract type

Poster

Section Type

AEM

368 - Genome-informed phenotyping of *Lysobacter* strains from Saskatchewan pea fields reveals antagonism against *Aphanomyces* and other root rot pathogens

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Root rot complex remains a major constraint to pea production in the Canadian Prairies, with *Aphanomyces euteiches* representing a particularly difficult component due to persistence in soil and limited effective management options. Members of the genus *Lysobacter* are recognized for producing antagonism-associated extracellular enzymes and antimicrobial compounds that support biological control of soil-borne pathogens in controlled conditions. Here, we integrate a genome-informed framework with targeted phenotyping to evaluate two *Lysobacter* strains isolated from Saskatchewan: *Lysobacter capsici* K-Hf-H2 and *Lysobacter gummosus* K-Be-H3. Whole-genome sequence information was used to guide a trait-focused interpretation of biocontrol potential, emphasizing functions consistent with extracellular lytic capacity and antimicrobial activity. Phenotypic screening was conducted primarily against *Aphanomyces*, with additional assays against *Rhizoctonia* spp., using in vitro antagonism assays. Both strains consistently inhibited pathogen growth in vitro. Diffusion-style assays further indicated secretion of inhibitory, diffusible compounds that suppressed pathogen development beyond the zone of direct contact, supporting a role for secreted factors in antagonism. In addition, time-lapse microscopy of *Aphanomyces* hyphal growth in the presence of *Lysobacter* revealed altered growth dynamics at the interaction front relative to untreated controls, consistent with direct inhibitory interactions. Together, these results demonstrate the utility of combining genome-informed expectations with scalable phenotyping and imaging to prioritize candidate *Lysobacter* strains for subsequent greenhouse and field validation in Prairie pulse systems.

Gen abstract type

Poster

Section Type

AEM

371 - Engineered Yeast-Based Therapeutics Can Target and Clear Pathogenic Gut Bacteria

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Food insecurity is one of the leading contributors to poor health outcomes in both adults and children, increasing the likelihood of heart disease, cancer, and even depression and anxiety. Poultry products can help provide affordable and nutrient-rich meat and eggs, and as such, are heavily relied on by families all around the world. However, challenges like disease can significantly impact the health of poultry birds, contributing to food scarcity. One of such diseases that notably affects these animals is necrotic enteritis, which arises from intestinal overgrowth of the *Clostridium perfringens* pathogen. This infection is associated with a high fatality rate in birds, costing the poultry industry approximately $6 billion annually in the US alone. As such, this significantly contributes to the rising costs of poultry food products and exacerbates food insecurity in vulnerable populations. The current solution for the necrotic enteritis disease uses antibiotics; however, prolonged exposure is associated with the development of antibiotic-resistant strains that can be transferred to humans and pose serious health concerns. In response to this, our group is engineering the probiotic yeast, *Saccharomyces boulardii,* as an alternative therapeutic for addressing necrotic enteritis disease. *S. boulardii* is an attractive probiotic because it thrives well in the gut environment, is a fast-dividing microbe, and can be used as a chassis for a broad range of antimicrobial peptides. Leveraging these characteristics, we aim to engineer this probiotic yeast to produce antimicrobial peptides that target *C. perfringens* in the gut and promote its clearance.

Gen abstract type

Poster

Section Type

AEM

373 - Growth Kinetics *Methylophilus methylotrophus* AS1 in Recycled Single Cell Protein Spent Media

Flavia Hirabayashi Cleto[1], Domenic Marano[1]

[1]University of Alberta

Spent fermentation media represents a substantial waste stream in bioprocessing and as fermentation scale increases, disposal of this liquid stream presents a growing economic and environmental burden. This work investigates whether spent fermentation media from the production of single cell protein (SCP) can be repurposed as a recyclable feedstock through targeted characterization and treatment.

SCP harvest material was subjected to a sequential treatment process consisting of flocculation and centrifugation for separation of the SCP product from the spent media, tangential flow filtration for spent media clarification, and granular activated carbon adsorption for the removal of residual organic compounds. The resulting liquid fractions were fully characterized to track changes in bulk composition and organic matter.

The results demonstrate highly effective removal of cell debris and residual organic material across flocculation, filtration, and adsorption treatments. Elemental analysis confirms that essential inorganic nutrients remain largely conserved throughout the process, with only minor alterations in select cation concentrations compared to fresh media.

Parallel shake-flask trials are currently being employed to compare microbial growth kinetics in fresh versus recycled media after each treatment step. Furthermore, growth assays will be performed to define maximum tolerable concentrations of common metabolic byproducts and nutrients that tend to accumulate during fermentation cycles. Together, these experiments aim to identify which treatment steps are necessary and sufficient to restore or maintain suitable media quality, and at what point additional processing may otherwise offer diminishing returns. Developing strategies to reuse this spent media serves to reduce the operational costs and environmental impact of SCP production, providing a circular approach to waste stream management.

Gen abstract type

Poster

Section Type

AEM

374 - Heat and Desiccation Resistance in Extraintestinal Pathogenic *Escherichia coli* (ExPEC) and Its Implications for Biosolids Waste Management

Ashley Chew[1, 2, 3, 4, 5]

[1]Norman Neumann, [2]Patrick Hanington, [3]Simon J.G. Otto, [4]Candis Scott, [5]Kanghee Ryu

Introduction: Previous studies have identified heat-resistant *E. coli* in wastewater systems, notably naturalized wastewater *E. coli* (NWEC) possessing the transmissible locus of stress tolerance (tLST). Recent evidence indicates that wastewater-derived extraintestinal pathogenic *E. coli* (W-ExPEC) also survive wastewater treatment and are heat resistant despite lacking the tLST, suggesting alternative mechanisms of heat tolerance. Since elevated temperatures are characteristic of biosolids treatment (i.e., composting), biosolids processing may represent a key step in the natural selection of heat-resistance in *E. coli*, enabling the persistence of pathogenic strains through thermal treatment. This study aimed to evaluate heat and desiccation resistance in W-ExPEC and NWEC to better understand pathogen survival mechanisms.

Methods: *E. coli* isolates from chlorinated sewage and wastewater effluents, including W-ExPEC strains, laboratory reference strains, and an NWEC strain, were suspended in matrices with various nutrient availability and exposed to 50-55°C for up to 9 hours. In some experiments, isolates were thermally acclimatized before heat shock exposure. Bacterial survival was quantified using a modified most probable number (MPN) spot assay. Desiccation tolerance was assessed over short- (24-48 h) and long-term (7-21 days) periods. Statistical significance was evaluated using one- or two-way ANOVA with Bonferroni post-tests.

Results: W-ExPEC and NWEC strains exhibited significantly greater heat resistance than laboratory control strains, surviving exposure to 55°C for up to 2 hours, whereas ATCC 25922 and clinical CFT073 were completely inactivated within 1 hour, the earliest time point measured. Notably, several W-ExPEC strains survived exposure to 52°C for more than 9 hours under certain conditions. Both W-ExPEC and NWEC strains also demonstrated substantial desiccation tolerance, persisting for up to 21 days. Slow thermal acclimatization increased W-ExPEC heat resistance by approximately 1,000-fold compared to immediate heat shock, indicating that acclimatization enhances thermal tolerance.

Significance: These findings demonstrate remarkable heat and desiccation resistance in ExPEC highlighting challenges for microbial inactivation during biosolids treatment. Gradual thermal acclimatization may further compromise treatment efficacy, raising concerns about the persistence of potentially pathogenic *E. coli* during biosolids processing and downstream environmental exposure through food and water.

Gen abstract type

Poster

Section Type

AEM

375 - Survival of Extraintestinal Pathogenic *Escherichia coli* Strains During Municipal Biosolids Treatment and Potential Implications for Food and Water Safety.

Ashley Chew[1, 2, 3, 4, 5]

[1]Norman Neumann, [2]Patrick Hanington, [3]Simon J.G. Otto, [4]Candis Scott, [5]Kanghee Ryu

Introduction: Extraintestinal pathogenic *Escherichia coli* (ExPEC) are increasingly being detected in treated wastewater and biosolids through culture-based and genomic studies, raising concerns about their persistence beyond conventional sanitary treatment processes. This data suggests that ExPEC may bypass or survive standard pathogen-reduction strategies, thereby increasing the risk of environmental dissemination and human exposure. Understanding the mechanisms that enable ExPEC survival during biosolids treatment is therefore critical for assessing treatment efficacy and public health risk.

Methods: A library of *E. coli* strains was isolated from municipal sludge/biosolid samples (anaerobic digested and thickened sludge). Purified isolates were confirmed to be *E. coli* by PCR (i.e., *uidA* gene) and by biochemical identification on a VITEK 2® Automated Bacterial Identification System. Virulence gene (VG) screening by PCR (*ibeA, fyuA, chuA,* and *iroN*) was done to identify potential ExPEC strains, and included screening for ST131 strains (a notable pandemic strain). Strains positive for virulence genes were subjected to whole-genome sequencing (WGS) followed by phylogenetic analysis and detailed VG characterization.

Results: 52 of 180 (28.9%) library isolates were positive for two or more ExPEC-associated virulence genes, with 9 strains (5.0%) containing all 4 virulence genes. Of these 52 strains, only four (7.7%) were ST131 positive, and repetitive extragenic palindromic PCR (rep-PCR) revealed that 19 of the 52 strains appeared clonally related, leaving 33 potential ExPEC strains (18.3%) to be genome sequenced. Genomic analysis revealed prominent ExPEC serotypes such as ST73, ST95, and ST131, and was closely related to clinically-relevant ExPEC strains with ANI scores greater than 99.8%. A more thorough genomic analysis on phylogenetic-relatedness and VG composition will be presented at the conference.

Significance: Treated municipal biosolids appear to contain *E. coli* strains with ExPEC-associated virulence genes, indicating that potentially pathogenic bacteria can persist through conventional sludge treatment processes and highlights the potential risks biosolids might pose as agricultural soil amendments for food production.

Gen abstract type

Poster

Section Type

AEM

376 - Bacteriophages suppress *Pseudomonas syringae* colonization of blueberry leaves

Philip Lauman[1], Cassidy Ball[1], Tongzhou Xu[2], Keiran Cross[3], Thomas Guy[3], Mark Lubberts[3], Melinda Ren[1], Rishi Burlakoti[4], Karen Fong[3], Xiangyu Deng[2], Siyun Wang[1]

[1]University of British Columbia, [2]University of Georgia, [3]Summerland Research & Development Centre, [4]Agassiz Research & Development Centre

Pseudomonas syringae is a widespread phytopathogen responsible for crop losses in virtually all economically relevant crop species including highbush blueberries, in which it is the causative agent of leaf blight. The cultivation of highbush blueberries is a major component of the agricultural sector in British Columbia, contributing roughly $280 million per annum to the local economy, but the same environmental conditions that favour cultivation simultaneously promote the proliferation and virulence of *P. syringae*. Existing management strategies rely heavily on copper-based treatments, which are increasingly limited by environmental concerns and the emergence of resistance, highlighting the need for novel approaches.

(Bacterio)phages, naturally occurring and self-replicating viruses that infect target bacteria while leaving commensals and plant cells unharmed, have emerged as an alternative solution. Although phages have shown promise as biocontrol agents against *P. syringae* in other crops, no phages specific to blueberry-associated strains have been characterized thus far, and the potential of phages to prevent leaf blight in blueberries therefore remains largely unexplored.

Here, we isolated 19 novel phages from agricultural environments across southwestern British Columbia using a panel of *P. syringae* strains derived from infected blueberry plants, and selected 12 candidates with simultaneously maximized host ranges and infectivity. The utility of these 12 phages was further validated through kinetic and environmental stability assays, while whole-genome sequencing was employed to confirm the absence of unfavourable genetic traits.

The antibacterial efficacies of individual candidate phages and five-phage cocktails were subsequently evaluated using *in vitro* killing assays. While antibacterial activity was observed in virtually all phage-strain pairs, complete inhibition was rare and efficacy was found to depend strongly on host susceptibility. Cocktail formulation provided modest improvements in antibacterial efficacy, primarily by enhancing suppression in strains with intermediate susceptibility to individual phages, but conferred limited benefits against broadly phage-resistant strains.

To assess performance in an agriculturally relevant context, phage cocktails were tested in an *ex planta* detached-leaf infection model. Substantial and persistent reductions in bacterial colonization were observed in phage-treated leaves relative to untreated controls, with most cocktails reducing bacterial densities below the limit of detection. Interestingly, antibacterial efficacy *in vitro* correlated strongly ($r = 0.64$; $p = 0.0007$) with performance in the detached leaf model, indicating that *in vitro* activity partially predicts phage suitability for biocontrol.

Together, these findings demonstrate that bacteriophages can be systematically selected and deployed to suppress *P. syringae* colonization of blueberry leaf tissues, and more broadly support the development of tailored phage-based strategies for agricultural biocontrol.

Gen abstract type Poster **Section Type** AEM

388 - Divergent Global Biogeography and Global Change Sensitivity of Phyllosphere Bacterial and Fungal Communities

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The phyllosphere is one of Earth's largest microbial habitats, yet broad-scale differences between bacterial and fungal communities remain poorly resolved. Here, we synthesized two global datasets from 123 studies to examine how phyllosphere bacterial and fungal communities vary across climatic, spatial and host-associated gradients, and how they respond to major global change factors (GCFs) including warming, drought, elevated CO_2 and nitrogen addition. Our analyses revealed clear bacteria–fungi divergence in both biogeographic patterns and environmental sensitivity. Bacterial diversity and community composition were more closely associated with climatic and spatial gradients, whereas fungal communities showed stronger associations with host-related variation. Bacteria and fungi also differed in their latitudinal patterns and in the relative importance of turnover and nestedness components of β-diversity. Responses to GCFs differed not only between bacteria and fungi but also across community metrics. In particular, changes in community composition were more broadly detected than changes in α-diversity, indicating that taxonomic reorganization may occur even when overall diversity appears relatively stable. These findings demonstrate that phyllosphere bacteria and fungi follow distinct global patterns and respond differently to environmental change, highlighting the need to consider microbial groups and community metrics separately when predicting phyllosphere microbiome responses under future global change.

Gen abstract type

Poster

Section Type

AEM

390 - Impact of a Milk Replacer-based Medium on Microbial Stabilization and Short Chain Fatty Acid (SCFA) Profiles in an in vitro Calf Hindgut Model

Farah Al Qudsi[1], Gisèle LaPointe[1], Gregory Higgins[1]

[1]University of Guelph

Developing an *in vitro* calf hindgut model offers a controlled platform for investigating gut microbiome modulation using feed additives, if the microbial population can be stabilized. During the pre-weaning period, calves consume milk replacer in combination with solid feed. Standardized media contain carbohydrates and proteins, but may not adequately reflect the composition of milk proteins and fat of the pre-weaned calf diet. This study aims to investigate whether a calf milk replacer-based medium supports microbial community stabilization in an *in vitro* calf hindgut system, compared to commercial medium used for the infant Simulator of the Human Intestinal Microbial Ecosystem (SHIME).

A calf fecal sample was used to mimic the calf hindgut in the Infors chemostat model under anaerobic conditions with controlled environmental factors including pH, temperature, and nutrient supply. Two chemostats were inoculated with a calf fecal slurry and operated for 28 days to attempt to reach steady state. Vessels were continuously fed either a formulated calf milk replacer (CMR) medium with total solids of 20 g/L, consisting of 4% monosaccharides, 7.5% disaccharide (lactose), 19% complex carbohydrates (16% potato starch, 3% corn starch), 24 % proteins (13% milk proteins, 8% plant protein) and 4.5% fat, or a commercial infant SHIME medium with 17 g/L total solids, composed of 6% monosaccharides, 18% disaccharides (12% lactose, 6% cellobiose), 12% complex carbohydrates (6% pectin, 6% corn starch) and 28% proteins (17% milk proteins, 11% plant protein). Samples were collected every 7 days to monitor metabolic stabilization using proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). Microbial composition was monitored using 16S ribosomal RNA gene amplicon sequencing.

SCFA concentrations and microbial community composition in the CMR-fed vessel showed microbial variability over the 28-day period, with repeated shifts in the relative abundance of dominant genera between sampling points and no consistent convergence toward a stable profile. In contrast, the commercial infant SHIME medium achieved stabilization of total SCFA concentrations by day 21, indicating the establishment of a more stable metabolic state. Butyrate was lower in the CMR-fed vessel than in the infant SHIME-fed vessel (5 compared to 15 mol%), corresponding to a reduced abundance of key butyrate-producing taxa such as *Ruminococcus*. This reduction might be associated with the starch-fat enriched composition of the CMR medium bedsides lower fermentable carbohydrates, which limit complex polysaccharide-driven fermentation and increase lipid-associated microbial groups. This shift in community structure might disrupt cross-feeding networks required for butyrate synthesis, particularly the transfer of acetate from primary fermenters to butyrogenic taxa. Consequently, carbon flow is redirected toward alternative fermentation pathways, including propionate production, leading to the observed increase in propionate levels and a metabolic shift in SCFA profiles.

These findings indicate that the commercial infant SHIME medium achieves microbial population stability while the CMR medium does not. Biofilm accumulation in the CMR-fed vessel might be the reason for the microbial instability. Further work is necessary to optimize the milk replacer medium by reducing total nutritional content to limit the overgrowth of fast-growing taxa, and reduce inhibitory metabolite accumulation, thereby promoting a metabolically stable microbial population.

Gen abstract type Poster

Section Type AEM

391 - A Sequencing-Based Approach for Typing Measles Virus from Wastewater

Amber Papineau[1], Jennifer Ali[1], Shayna Giesbrecht[1], Veronica Carroll[1], Gajuna Mathiyalagan[1], Michael McKay[2], Joanne Hiebert[1], Chrystal Landgraff[1]

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Wastewater-based surveillance of pathogens was modernized and widely deployed during the SARS-CoV-2 pandemic to study viral circulation at the community level. Municipal wastewater aggregates pathogen signals from multiple sources, resulting in derived sequences that can represent multiple viral lineages simultaneously. As a result, genetic analysis of pathogens in wastewater typically requires specialized laboratory and bioinformatic methodologies designed to deconvolute heterogenous sequence data.

Measles cases have been increasing across the globe. To determine whether wastewater surveillance could be utilized to inform community-level diversity of Measles virus (MeV), we developed and validated methods for the genetic analysis of measles virus in wastewater. Here, we describe a bioinformatic pipeline intended for the analysis of Illumina sequencing data. The pipeline performs genotype-level deconvolution and further assigns distinct sequence identifiers (DSids).

These methods were validated in silico using artificially mixed datasets and further evaluated using a pilot study of municipal wastewater samples. Finally, we demonstrate the targeted N gene amplicons processed by the bioinformatic pipeline can determine MeV genotype(s) from wastewater and assign DSids to low complexity wastewater samples.

Gen abstract type

Poster

Section Type

AEM

392 - Microbial consumption of H_2 and impacts on metal infrastructure corrosion - implications for underground H_2 storage

Taylor Surcon[1], Xavier Lewis[1], Peter Bell-Scholz[1], Lisa Gieg[1]

[1]University of Calgary

Hydrogen is an energy carrier that has received increased attention as a carbon neutral energy source because its use only releases water vapour as a by-product. As H_2 is a low molecular weight compound, it dissipates quickly into the atmosphere making it difficult to store in a cost-effective manner. However, H_2 can be stored below ground to maximize scalability potential in aquifers, spent oil reservoirs, or salt caverns. Microorganisms can inhabit these environments and potentially metabolize stored H_2, transforming it into other gasses such as methane and sulfide. These other products can contaminate the H_2 source and sulfide can be damaging to infrastructure. Therefore, it is important to understand microbial metabolism occurring in these environments and whether any other negative effects like microbiologically influenced corrosion (MIC) of associated infrastructure can occur, as well as identifying the microbes responsible for these effects.

Our research is focused on investigating the microbial consumption of H_2 under high salinity conditions associated with salt cavern storage in order to determine whether this metabolism could also lead to increased corrosion of metal infrastructure. To carry out this research, sulfate-reducing and methanogenic cultures were established from oilfield produced waters with salinities of up 1.9 M NaCl and at a temperature of 30ºC using H_2 as a nutrient source. Active sulfate-reducing or methanogenic H_2-using enrichments were then used to carry out MIC experiments wherein carbon steel beads were added as the metal source. Initial results showed increased metal weight loss in H_2-amended cultures compared to H_2-free cultures, suggesting that the microbial consumption of H_2 increased the threat for corrosion. Our current work has been focused on establishing and scaling up the initial cultures and monitoring H_2 consumption due to sulfide production and methanogenesis using the sulfide barium chloride assay and gas chromatography, respectively. These incubations have demonstrated that microorganisms inhabiting saline environments do have the potential to consume H_2, produce sulfide or methane, and carry out metal corrosion. Amplicon sequencing analysis based on the 16S rRNA gene of these cultures revealed a high abundance of known sulfate-reducers and methanogens such as *Desulfomicrobium*, *Desulfocurvus*, and *Methanoculleus*. Thus, the microbial community results corroborate the experimental evidence of these metabolisms.

Overall, the outcome of this research is to provide more information on the microbial processes like MIC that can affect long-term H_2 storage so that hydrogen can be used as an effective energy source in the future.

Gen abstract type

Poster

Section Type

AEM

393 - Metagenomic Dissection of Microbial Community Structure and Metabolic Potential Across a Natural Methane Gradient in Anaerobic Environments Contaminated with Hydrocarbons

Andrés Merino-Restrepo[1], Iram Afzal[1], Alsu Kuznetsova[1], Tariq Siddique[1]

[1]Department of Renewable Resources, University of Alberta, Edmonton, Alberta T6G 2G7, Canada

Anaerobic sediments contaminated with hydrocarbons can harbor complex microbial communities that drive both hydrocarbon biodegradation and greenhouse gas (methane) production. These sediments contain fine tailings, process-affected water, and residual hydrocarbons, making them relevant systems for studying the intersection of hydrocarbon biodegradation and environmental reclamation. Understanding the metabolic architecture of the microbial communities present in these sediments is critical for promoting hydrocarbon mineralization while suppressing methane emissions, central goals for responsible tailings reclamation.

This study aimed to: (1) characterize microbial community structure across locations and depths in oil sands process-affected water and contaminated anaerobic sediments exhibiting contrasting methane outputs (high- and low-methanogenic groups); (2) identify metabolic pathways and functional marker genes supporting methanogenesis and anaerobic hydrocarbon degradation; and (3) establish a genomic baseline for future comparison with laboratory incubations under different electron-acceptor conditions. We hypothesized that high-methanogenic locations are enriched in pathways for methanogenesis and syntrophic hydrocarbon degradation relative to low-methanogenic locations; however, methanogenic output reflects not only catabolic pathway presence but also community organization.

Eleven metagenomes were grouped into high-methanogenic (HM; n = 5) and low-methanogenic (LM; n = 6) communities based on in-situ methane production. Two custom Bash/Python scripts were developed to implement a fully automated, reproducible Anvi'o v9 workflow. The upstream script performed quality-controlled co-assembly with MEGAHIT, contig database generation, HMM profiling, KOfam/COG24 annotation, Bowtie2-based read recruitment, merged group profiling, and MetaBAT2 binning. The downstream script executed collection verification, KEGG module completeness estimation (community- and bin-level), targeted KEGG pathway map generation (methane, sulfur and nitrogen metabolism, and benzoate and toluene degradation pathways), locus export for 13 functional marker genes, SCG-based taxonomy, phylogenomic tree inference using Bacteria_71 and Archaea_76 marker sets, variability profiling, and metabolic enrichment analysis comparing HM versus LM bins.

Both groups exhibited comparable per-sample genomic complexity, with HM averaging ~143.6 Mb and ~177,900 predicted genes per metagenome and LM averaging ~141.5 Mb and ~175,300 predicted genes per metagenome, indicating broadly equivalent genomic richness across groups. *Pseudomonadota* dominated both groups; *Desulfobacterota* was slightly elevated in HM, and *Halobacteriota* was more prominent in HM, consistent with greater archaeal methanogenic activity. Methanogenesis marker genes (mcrA, mcrB, mcrG) were enriched in HM (3.2 vs. 0.7 hits per sample), while aerobic methane oxidation markers (pmoA, pmoB, pmoC) showed consistently stronger signals in LM (10.3 vs. 7.2 hits per sample); this pattern likely reflects ecological differences in the balance between methanogenesis and aerobic methane oxidation at the sediment-water interface. Sulfur (dsrA, dsrM, dsrK) and nitrogen (nifH, nifD) cycling genes were present in both groups. Both communities encoded complete or near-complete KEGG modules for aromatic hydrocarbon degradation, including benzoate and toluene, and for aliphatic hydrocarbon catabolism via fatty acid β-oxidation.

These results demonstrate that differential methanogenic output in hydrocarbon-contaminated anaerobic sediments is governed by community syntrophic organization and acetoclastic guild structure rather than by the presence or absence of catabolic genes, a finding that has direct implications for engineering electron-acceptor conditions capable of selectively suppressing methanogenesis while preserving hydrocarbon biodegradation capacity, and that provides a critical genomic baseline for such comparative studies.

Gen abstract type Poster

Section Type AEM

SPEAKERS & ABSTRACTS NOT SCHEDULED FOR POSTERS/ RÉSUMÉS & NON PRÉVUS POUR LES AFFICHES

This section summarizes abstracts submitted to the conference that are not scheduled for poster sessions (Abstracts only). These abstracts also include those submitted for invited talks and abstracts selected by conference symposium chairs for talks in various symposia sections. Abstracts are numbered according to their original submission order.

Cette section résume les résumés soumis à la conférence qui ne sont pas prévus pour des sessions d'affiches (résumés uniquement). Ces résumés incluent également ceux soumis pour des conférences invitées et ceux sélectionnés par les responsables des symposiums de la conférence pour des conférences dans diverses sections de symposiums. Les résumés sont numérotés selon leur ordre de soumission initial.

CSM Keynote and Award Lectures/ Conférences de Remise des Prix SCM

415 - Pushing the boundaries of cryo-microscopy to gain insight into host-microbe interactions



Ariane Briegel[1]

[1]Institut Pasteur

How are bacterial cells able to actively seek out their preferred environmental niches? How can they evade toxins and predators? How do they interact with phages, each other and their host tissue on a structural level?

Cryo-electron tomography (cryo-ET) is our key research tool to gain insight into the structure and function of the molecular complexes involved in these behaviors. This technique allows us to directly study microbes in their native state at resolutions capable of visualizing individual proteins. However, studying how microbes interact in more complex environments, such as host tissue, is still dauntingly difficult. Here we apply a large volume processing pipeline to study a naturally simplified host microbe system where one organism, the Hawaiian bobtail squid, interacts with only one species of bacteria. Once the squids hatch, the light-producing bacterium *Vibrio fischeri* in the surrounding seawater migrate toward and into the squid's internal light organ, where they colonize and maintain a light-producing population for the lifetime of the squid. This makes the animal invisible for predators at night, as the animal is camouflaged against the moonlight shining from above. This binary host-microbe interaction is an ideal system to begin to understand the structural interplay between these organisms at the nanoscale.

Section Type MGCM

Invited Keynote Speaker June 16, 2026

225 - Comparative Mapping of Antifungal Resistance Mutations in the Main Antimicrobial Target of Fungal Pathogens



Camille Bédard[1, 2, 3, 4, 5, 6]

[1]Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, Canada, [2]Département de Biochimie, de Microbiologie et de Bio-informatique, Faculté des Sciences et de Génie, Université Laval, Québec, Canada, [3]Le regroupement québécois de recherche sur la fonction, l'ingénierie et les applications des protéines (PROTEO), Québec, Canada, [4]Centre de Recherche sur les Données Massives (CRDM), Université Laval, Québec, Canada, [5]Centre de Recherche en Infectiologie (CRI), Université Laval, Québec, Canada, [6]Institut Intelligence et Données (IID), Université Laval, Québec, Canada

Fungal pathogens pose a growing threat to human health, agriculture, and natural ecosystems. Because a small number of antifungal classes are available, antimicrobial resistance (AMR) evolution threatens treatment efficiency and disease control. To address this challenge, a thorough understanding of resistance mutations across fungal species and antifungals is critical.

First, we assembled the FungAMR database, which contains 35,792 manually curated entries across 208 drugs and 95 fungal species. The analysis of this resource revealed mutations that confer resistance across dozens of species that diverged over hundreds of millions of years, suggesting that resistance mutations may be conserved across species. In addition, widespread cross-resistance was observed between antifungal compounds used in clinical and agricultural settings.

To experimentally test and quantify these observations on a large scale, we focused on azoles, the most widely used class of antifungals in both clinics and agriculture. Since amino acid substitutions in the essential and highly conserved azole target enzyme Erg11 are the most common resistance mechanism, we comprehensively characterized resistance-conferring variants in *Candida albicans* and the model yeast *Saccharomyces cerevisiae* using systematic mutagenesis.

In *C. albicans*, an average of 1,000 resistance-conferring variants per azole were identified. Nearly 90% of these variants conferred resistance to multiple azoles, indicating extensive cross-resistance. As expected, cross-resistance is higher within clinical or agricultural azoles, but 40% of variants resistant to agricultural azoles also conferred resistance to a clinical azole.

Then, the comparative analysis of the mutational effects between species revealed both conserved and species-specific resistance patterns, indicating only partial conservation of resistance mutations. Notably, *S. cerevisiae* has access to 50% fewer resistance mutations than *C. albicans*. However, ~80% of the smaller set of resistance-conferring mutations in *S. cerevisiae* are transferable to *C. albicans*. One hypothesis is that the lower intrinsic susceptibility of *S. cerevisiae* Erg11 constrains the evolution of acquired resistance.

To test this hypothesis, intrinsic Erg11 susceptibility was evaluated across 12 additional fungal species. Despite strong sequence conservation, substantial interspecific differences with a strong phylogenetic signal were observed. Ongoing work includes the characterization of Erg11 from 16 additional species, the reconstruction of eight ancestral sequences to identify residues associated with reduced susceptibility and experimental validations.

Together, these results provide a framework to improve resistance prediction in fungal pathogens and guide the development of antifungal drugs overcoming both acquired and intrinsic resistance.

Section Type: MGCM

Armand Frappier Award Lecture, June 18, 2026

From Roots to Wings: Understanding Microbial Evolution for Sustainable Plant Protection



Edel Pérez-López[1]

[1]Université Laval, Quebec, QC

Dr. Edel Pérez-López is an Associate Professor at Université Laval and holds a Canada Research Chair in Insect Vector Invasions and Emergent Plant Diseases within the Department of Plant Sciences. His research sits at the interface of microbiology, plant pathology, and entomology, with a strong focus on understanding how microbial pathogens and insect-associated microbiomes shape plant health in agricultural systems. Dr. Pérez-López's training includes molecular microbiology, plant-microbe interactions, and pathogen genomics. He completed his PhD in Ecology and Biotechnology at Universidad Veracruzana (Mexico), followed by postdoctoral training at Auburn University (USA) and the University of Saskatchewan. Since establishing his research program at Université Laval in 2020, he has developed an internationally recognized program focused on the molecular biology and evolution of plant-associated microbes, particularly the obligate biotrophic pathogen *Plasmodiophora brassicae* and insect-transmitted phytoplasmas. His work integrates microbiology with ecological and computational approaches to develop sustainable strategies that reduce pesticide use and improve crop resilience. His contributions have been recognized through several national and international awards, including the Hubert-Reeves Prize (Prix du Québec), the Outstanding Young Scientist Award from the Canadian Phytopathological Society, the William Boright Hewitt Award from the American Phytopathological Society, and the Equality, Diversity and Inclusion Prize from the Microbiology Society. He is also a strong advocate for equity, diversity, and inclusion in science, actively contributing to initiatives that promote a more inclusive and supportive microbiology community like Pride in Microbiology Network.

Section Type AEM

Thermo Fisher Award Lecture & Award Recipient June 17, 2026

407 - Cryomicrobiology: microbial life in the extreme cold on Earth and possibly beyond.



Lyle Whyte[1]

[1]McGill University, Montreal

This presentation will describe highlights of our research examining microbial biodiversity / activity / ecology in unique polar cryoenvironments which inform on the cold temperature limits of microbial life on Earth, climate change impacts, and guide the search for life on other cold planetary bodies like Mars, Europa, and Enceladus. Permafrost represents an extreme cryoenvironment yet harbours microbes with some the coldest growth temperatures yet discovered, including *Planococcus halocryophilus*. The most rapid warming on Earth is occurring in the Arctic and there is a fear that GHG emissions from melting permafrost soils will create a positive feedback loop of further GHG emissions. Results from our research have detected active microbial ecosystems present in high arctic permafrost environment and GHG fluxes originating from permafrost mineral cryosols. Results from our recent research have detected active microbial ecosystems present in surface ice from the Devon Ice Cap, and trace gas (H_2, CO, CH_4) metabolisms in permafrost and deglaciated soils on Axel Heiberg Island. The Canadian High Arctic features several anoxic, hypersaline cold springs including Lost Hammer Spring, which perennially discharges anoxic, subzero brines (−5°C; 24% salinity) through ~600 m of permafrost. A combination of metagenome assembled genome (MAGs), single-amplified genome (SAG) analyses in parallel with metatranscriptome analysis identified the dominant taxonomic and metabolic diversity in the hypersaline (~25% salinity), subzero (-5ºC), anaerobic Lost Hammer spring system and revealed a a rare surface terrestrial habitat supporting a predominantly lithoautotrophic active microbial community. We interpreted the identified active microbial ecosystem members and metabolisms - sulfide oxidation, sulfate reduction, anaerobic oxidation of methane, and oxidation of trace gases (H2, CO2) - as potential life forms that could exist in very cold and saline environments on Mars and the icy moons.

Section Type AEM

Murray Career Achievement Award Lecture & Award Recipient June 19, 2026

Symposium 1 Abstracts/ Résumés du symposium 1

400 - Fermented plant foods in sustainable and health-beneficial food systems

Michael Gänzle[1]

[1]University of Alberta

Agri-food systems in North America and other developed countries aim to shift from animal protein foods to whole plant foods. An increased consumption of plant protein at the expense of animal protein can increase the sustainability of agri-food production systems as well as the health of consumers. This presentation will discuss the role of fermented plant foods in the production of sustainable and health-sustaining foods. To investigate the sustainability of fermented whole plant foods, emphasis is placed on life cycle assessments to inform whether fermented plant foods provide sustainable alternatives to meat and dairy products. Results document that prioritising plant-based products over animal products can be a solution to improve the sustainability of our food system. However, fermented plant foods that are formulated from purified plant-based ingredients such as purified protein, starches, fats and other ingredients or additives all but eliminates or even reverts the improved sustainability of whole plant foods when compared to meat and (fermented) dairy products. Likewise, multiple studies document that a diet rich in (fermented) whole plant foods with minimal use of refined ingredients is health beneficial while foods that are formulated with refined ingredients and additives, often referred to as “ultra-processed foods” are not, irrespective of fermentation steps. Choosing whole plant foods with minimal addition of refined ingredients and additives is thus the best solution to reduce the environmental footprint of food production and to attain health benefits. Fermentation is a key tool to reduce the need for ingredients and additives. In addition, fermentation presents the unique ability to moderate off-flavours and bitterness associated with plant material and turn it into a more appealing and palatable product.

Section Type AEM

Invited Speaker Symposium 1 - Microbes and the Future of Food June 17, 2026

384 - Through the Looking-Glass, and What Food Microbiologists Found There

Siyun Wang[1]

[1]The University of British Columbia

Every year, approximately 4 million Canadians are affected by foodborne illnesses. This presentation explores the complexities and recent advances in controlling foodborne pathogens across the farm-to-fork continuum, using *Salmonella enterica*—the world's leading cause of diarrhoeal disease—as a key example. The presentation will focus on biocontrol strategies and highlight best practices for optimizing microbial food safety within a One Health framework.

Section Type AEM

Invited Speaker Symposium 1 - Microbes & the Future of Food June 17, 2026

233 - Modelling interactions between *Listeria monocytogenes* and *Lactococcus lactis* A5

Denise Cao[1], Veronica Guariglia[1]

[1]Western University

Listeria monocytogenes (*Lm*) is a Gram-positive foodborne pathogen and the causative agent of listeriosis. Listeriosis is a rare, but serious condition that has a 20-30% mortality rate and almost always requires hospitalization. For this reason, controlling *Lm* contamination in food processing environments is a crucial consideration for food safety. A natural method to control *Lm* in foods is through bacteriocins, small antimicrobial peptides produced by bacteria to inhibit other similar bacteria. Bacteriocins are commonly found in the food environment from bacteriocin producers of the food microbiome, often lactic acid bacteria. However, whether *Lm* modulates producer strains of the food microbiome is currently not well understood. For this reason, we sought to investigate how *Lm* interacts with these bacteria. We hypothesize that *Lm* interacts with bacteriocin producers of the food microbiome to facilitate survival in food environments. Here, we investigated the interaction between *Lm* and *Lactococcus lactis* A5, a lactic acid bacterium and bacteriocin producer, through two different angles. First, an indirect coculture system was used to characterize the effect of *L. lactis* contact-independent interactions on *Lm* growth. RNA-seq of *Lm* in coculture revealed activation of the cell-envelope stress response and widescale metabolic changes, suggesting that *L. lactis* dramatically alters the coculture environment through the production of acid and potential bacteriocins, and the consumption of oxygen and preferred sugars. Second, the ability of *L. lactis* to inhibit *Lm* in an in-situ cheese model was also investigated. Environmental factors of the food environment, such as alternative carbon sources and fat content, have been shown to decrease the efficacy of bacteriocin treatment. Using a 10g fresh cheese model, we found that a protective starter culture of *L. lactis* A5 inhibited the growth of *Lm* at room temperature and refrigerated conditions. Understanding how *Lm* interacts with bacteriocin producers will not only provide insight into how *Lm* survives in polymicrobial food environments, but can also inform alternative strategies to food safety such as protective cultures. In the future, this work can be further expanded to investigate how *Lm* grows in more complex microbial environments, including with bacteria found in other microbiomes.

Section Type: AEM

Abstract selected for Symposium 1 - Microbes & the Future of Food June 17, 2026

284 - Multi-Omics Profiling of 24-Month Spontaneous Beer Fermentations: Early Seasonal and Batch Variability Drive Late Metabolite Heterogeneity

Shane Carey[1], Sheri Schmidt[1], Maanasa Mudoor Sooresh[1], Paulina de la Mata[1], James Harynuk[1], Joseph Sebastian[1], Benjamin P Willing[1], Benjamin Bourrie[2]

[1]University of Alberta, [2]MacEwan University

Spontaneously fermented beer is the product of complex microbial interactions that are highly sensitive to initial environmental conditions. These conditions are leveraged to produce an incredibly complex and variable beer that is quickly growing in popularity. Within these spontaneous fermentations, general community assembly is well-understood, but factors such as seasonality and batch to batch variation have not been explored in-depth. To address these unknowns this study incorporated shotgun-metagenomic sequencing, volatile metabolite profiling via GC×GC-TOFMS, and organic acid profiling via HPLC on 22 barrels from multiple batches and seasons at seven time points in a two year fermentation. Altogether we have data on over 120 timepoints that will help us elucidate differences between and within spring, summer, and fall fermentations. As anticipated, community assembly from our genomic dataset was consistent with established patterns from Lambic beer, however metabolomic data displayed high levels of heterogeneity that was not clearly explained by microbial profiles. Fermentation age was a large driver of difference in microbial profiles, i.e., early timepoints were closely associated with batch and seasonality but converged as the fermentations reached maturity. Metabolite profiles, however, remained seasonally distinct. Wort acidity also appears to be important for fermentation success, as barrels with a pH range of 4.7-5.4 had extended enterobacterial phases and did not establish a population of lactic acid bacteria, which are usually common to all spontaneous beer fermentations. These data suggest that starting conditions and early microbial communities impact metabolite production in late stage fermentations, regardless of later stage homogeneity. In order to elucidate the seasonal and batch variations from our datasets we are developing metabolic models for all fermentation timepoints to better understand how key taxa contribute at different stages of fermentation. These models enable us to map the different genetic pathways that are active across different barrels, batches and seasons. Additionally, we are leveraging 100s of high quality metagenome assembled genomes to determine the impact of strain-level diversity on fermentation outcomes. Because we have multiple high quality genomic bins for each of our most abundant organisms, e.g., *Pediococcus*, *Saccharomyces* and *Brettanomyces*, we are able to determine contributions at the strain level. Furthermore, all sampled barrels in this study are first-fill, and/or have not been used for spontaneous beer fermentation, which challenges the need for a back-slopping effect as a necessary inoculant for successful fermentations. This implies that common taxa observed across most spontaneous fermentations have greater ecological fitness within these environments, and are not abundant simply because of a high initial microbial load. Altogether, these data greatly advance our knowledge of microbial ecology in spontaneously fermented foods.

Section Type: AEM

Abstract selected for Symposium 1 - Microbes & the Future of Food June 17, 2026

312 - Multidimensional Engineering of Yeasts for Waste-Stream Feedstock Valorization

Mohamed Nasr[1], Radhakrishnan Mahadevan[1]

[1]BioZone, Dept. of Chemical Engineering and Applied Chemistry, University of Toronto, Ontario, Canada

Waste-stream feedstocks represent an underutilized resource for sustainable biotechnology, yet their efficient conversion into diverse valuable products remains a fundamental challenge. Here, we engineer the model yeast *Saccharomyces cerevisiae*, leveraging its industrial robustness and generally recognized as safe (GRAS) status, to develop platform strains that convert starchy agrifood waste into industrially relevant molecules.

Our approach integrates iterative rounds of CRISPR-mediated genome editing with protein engineering to enable non-native substrate utilization through improved secretion of heterologous amylases. We combine targeted gene overexpression and deletion with a multi-pronged protein engineering strategy integrating machine learning-guided, semi-rational, and random mutagenesis. These efforts generate strains with orders-of-magnitude increases in starch hydrolytic activity compared to the parent strain.

Using these engineered strains, we demonstrate the production of the biofuel ethanol and are extending this platform toward the production of the polymer building block lactic acid, as well as milk proteins for human nutrition and animal feed, all of which are of strong industrial interest.

Together, our work demonstrates how coupling genome and protein engineering can unlock yeast biomanufacturing platforms from low-cost, renewable feedstocks.

Section Type: AEM

Abstract selected for Symposium 1 - Microbes & the Future of Food June 17, 2026

Symposium 2 Abstracts/ Résumés du Symposium 2

398 - Finding 'needles in the haystack' central to anaerobic digestion: Uncovering novel syntrophic organisms using activity targeted 'omics

Ryan Ziels[1]

[1]The University of British Columbia

Microbes are the workhorses behind engineered bioprocesses such as anaerobic digestion; yet, a majority of microorganisms inhabiting these systems remain uncultivated. This conundrum is particularly pronounced for anaerobic syntrophic consortia, which are difficult to study because they are often slow-growing and rare. Yet, these organisms are important drivers of carbon turnover in anaerobic environments, often accounting for most of the methane (i.e., biofuel) production in such ecosystems. This knowledge gap of the microbial ecophysiologies driving engineered bioprocesses hinders our ability to accurately model ecosystem behavior and optimize system performance, such as for biofuel generation in biogas systems.

Here, I will discuss a novel molecular technique that addresses this challenge to quantitatively resolve microbial activity in rare and uncultivated consortia by combining bioorthogonal non-canonical amino acid tagging (BONCAT) and fluorescence-activated cell sorting (FACS) with ultra-low input metaproteomics and protein-based stable isotope probing (protein-SIP). We demonstrated this approach by feeding acetate to anoxic microcosms inoculated with biomass from a full-scale biogas facility and tracking protein expression profiles. Proteins from metabolically active members were selectively targeted with BONCAT using FACS, or by direct pulldown of proteins tagged with biotin using click chemistry. By utilizing BONCAT to enrich newly translated proteins and remove ‘old’ proteins from non-active microorganisms, we considerably amplified the detection of isotopically labelled peptides in the SIP metaproteomes. Specifically, BONCAT-enabled metaproteomes were dominated by the activity of a so-far uncharacterized syntrophic bacterium expressing a previously hypothesized novel pathway for syntrophic acetate oxidation involving the glycine cleavage system. Looking forward, this approach can be applied to other microbial ecosystems to link genomic identity with in situ metabolic function, a crucial challenge in the field of microbial ecology.

Section Type AEM

Invited Speaker Symposium 2 – Microbes & Energy June 17, 2026

399 - Metabolic strategies in chain-elongating bacteria for octanoic acid production from food waste

Christopher Lawson[1, 2]

[1]Chemical Engineering & Applied Chemistry, [2]University of Toronto

Anaerobic fermentation of organic wastes represents a promising route for sustainable production of fuels and chemicals, while reducing greenhouse gas emissions and landfill burden. Among these approaches, microbial chain elongation has emerged as a powerful platform for converting heterogeneous waste streams into C6-C8 medium-chain carboxylic acids (MCCAs), including hexanoic and octanoic acid. These products have broad industrial applications as antimicrobials, surfactants, feed additives and precursors for fuels, polymers and other oleochemicals. Because MCCAs are currently sourced primarily from palm kernel and coconut oil, waste-derived production could additionally reduce pressure on environmentally destructive agricultural supply chains.

MCCAs are produced by chain-elongating bacteria (CEB), a unique guild of strict anaerobes. Although all characterized CEB use reverse β-oxidation (RBO) to synthesize butyrate (C4), hexanoate (C6) and/or octanoate (C8), substantial variation exists in product selectivity across organisms and growth conditions. Understanding the metabolic and ecological basis of these differences remains a major challenge for improving MCCA yields, controlling product distributions and developing robust industrial bioprocesses.

In this presentation, I highlight recent studies from our lab uncovering fundamental metabolic strategies used by CEB to drive MCCA production. First, I describe how distinct acetate utilization strategies differentiate MCCA-producing CEB from organisms that solely produce the less valuable short-chain carboxylate butyrate. Next, I show how a novel genus of CEB drives octanoic acid production from amino acid- and lactate-rich waste streams through modulation of Stickland fermentation and RBO metabolism. Finally, I demonstrate how these physiological trade-offs are shaped by the substrate specificity and kinetic properties of key enzymes in the RBO pathway, and how recently developed genetic engineering tools can be used to further improve MCCA production. Together, these efforts represent important steps toward the domestication of this largely untapped group of anaerobic bacteria for sustainable biomanufacturing and resource recovery.

Section Type AEM

Invited Speaker Symposium 2 – Microbes & Energy June 17, 2026

405 - Deciphering the Complex Microbiology of Anaerobic Digestion for Energy Recovery

Camilla Nesbø[1], Torsten Meyer[1], Minqing Ivy Yang[1], Elizabeth Edwards[1], Courtney Toth[1, 2, 3]

[1]University of Toronto, [2]Northern Alberta Institute of Technology, [3]University of Alberta

Anaerobic digestion (AD) is an effective strategy for converting organic-rich industrial waste streams, including pulp and paper effluents, into methane and CO_2. AD also produces 80-90% less biomass than conventional aerobic treatment processes, reducing downstream costs associated with sludge disposal. Despite these advantages, only ~10% of Canadian pulp and paper mills have incorporated AD systems into their wastewater treatment plants. A major adoption challenge is that AD is governed by complex and largely uncharacterized microbial communities whose dynamics are difficult to predict, optimize, and control. This presentation will highlight recent laboratory enrichment studies, DNA sequencing efforts, and full-scale reactor investigations aimed at better understanding the microbial communities responsible for converting lignocellulosic waste into methane. Notably, anaerobic digesters appear to harbor distinct microbial “modules” spanning all trophic stages of AD, with some modules associated with stable, high-performing reactors and others emerging in response to perturbations. These insights may help inform predictive monitoring of AD health, as well as the development of new engineering strategies (e.g., staged digestion, bioaugmentation) to improve process stability and energy recovery.

Section Type AEM

Invited Speaker Symposium 2 – Microbes & Energy June 17, 2026

349 - Conversion of methane and methanol to isoprenoids using methanotroph *Methylomicrobium album* BG8

Shibashis Das[1], Lisa Stein[1], Dominic Sauvageau[1]

[1]University of Alberta

Methane and methanol are inexpensive and sustainable carbon feedstocks that can be used by methanotrophic bacteria as sole sources of carbon and energy and can be converted into value-added products. On the other hand, isoprenoids are valuable compounds that can be used as directly or as precursors for high performance fuels, flavoring agents, and pharmaceuticals, but are not readily produced by methanotrophs. In this work, the methanotroph *Methylomicrobium album* BG8 is modified to convert methane and methanol into beta-pinene, a simple isoprenoid. *M. album* BG8 contains the doxp pathway, which produces isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), two isoprenoid precursors, but it lacks the genes geranyl pyrophosphate synthase (GPPS) and pinene synthase (PS), which are required to transform these compounds into beta-pinene. In addition, work in other bacterial systems suggests low expression of the native DOXP pathway, leading to a potential bottleneck in generating large quantities of isoprenoids. In this work, conjugation was used to modify *M. album* BG8 with plasmids for high expression and low expression levels. A library of constitutive and inducible promoters was developed using green fluorescent protein (GFP) and nanoluc as markers. Co-transformation of high and low expression vectors was performed to improve the flux through the doxp pathway and the synthesis of beta-pinene. In particular, we generated two versions of the pinene expression operon by using native DXS (DOXP synthase) and DXS from *E. coli* K12. *M. album* BG8 transconjugants were cultured in bioreactors under batch and fed-batch operation to test isoprenoid production. This work provides a basis for the production of isoprenoids from methane and methanol by bacteria, an approach with great economic and environmental potential.

Section Type AEM

Abstract selected for Symposium 2 – Microbes & Energy June 17, 2026

Symposium 3 Abstracts/ Résumés du Symposium 3

404 - Uncovering cryptic antibiotic response mechanisms and new antimicrobials for MRSA wound infections

Omar El-Halfawy[1]

[1]University of Regina

Standard *in vitro* antimicrobial susceptibility testing (AST) using Mueller-Hinton broth (MHB) does not reflect infection-site conditions, and its results often do not correlate with therapeutic outcomes. We compared the antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA), a common chronic wound pathogen, in simulated wound fluid (SWF) resembling wound exudate versus MHB, revealing discordant AST results across six of nine tested antibiotic classes. The most significant were 128-fold increased resistance to tetracyclines and 256-fold sensitization to β-lactams in SWF. Tetracycline resistance was mediated by MntC, an extracellular manganese-binding protein, whereas β-lactam sensitization was driven by cell envelope remodelling in SWF. *Galleria mellonella* wound infection results matched the SWF susceptibility phenotypes, suggesting SWF better predicts *in vivo* wound infection therapeutic outcomes. To identify inhibitors of the MntC-mediated tetracycline resistance in the wound exudate, we conducted a high-throughput chemical screen in the presence of sub-inhibitory doxycycline in SWF. We discovered OES1-085, which synergized with doxycycline in SWF against the wild-type but not an *mntC*::Tn mutant, with no synergy observed in MHB against either strain. These comprehensive phenotypic and mechanistic insights into MRSA antibiotic responses under wound-infection-mimetic conditions, with direct *in vivo* validation, uncover a potential antibiotic adjuvant target and lead compound and may guide improved MRSA wound-infection therapy.

Section Type: I&I

Invited Speaker for Symposium 3 – Antimicrobial Resistance June 17, 2026

119 - Unlocking the Therapeutic Potential of Glucosamine-6-Phosphate Synthase (GlmS) by Overcoming Intrinsic Resistance

Georgina Cox[1]

[1]University of Guelph

The hexosamine biosynthetic pathway generates UDP-N-acetylglucosamine (UDP-GlcNAc), an indispensable building block for peptidoglycan, lipopolysaccharide (LPS), and teichoic acid synthesis in bacteria. Glucosamine-6-phosphate synthase (GlmS), also known as glucosamine-fructose-6-phosphate aminotransferase (GFAT), catalyzes the first committed step of this pathway and is highly conserved across all cellular life forms. In prokaryotes, GlmS is essential for growth when exogenous amino sugars are absent; host-derived glucosamine cannot sustain bacterial proliferation. Consequently, GlmS exhibits many attributes that make it an attractive antibiotic target.

Despite the identification of both natural and synthetic inhibitors that inhibit purified GlmS enzymatic activity, none have been able to suppress the growth of Gram-negative bacteria. The underlying cause is the sophisticated regulatory network governing GlmS levels, which varies among bacteria. In the Enterobacteriaceae family, regulation relies on a conserved small-RNA (sRNA) system comprising GlmZ and its mimic GlmY. When GlcN6P levels fall, for example following chemical inhibition of GlmS, these sRNAs upregulate GlmS synthesis, thereby conferring intrinsic resistance to the inhibitors.

In this presentation, I present an adjuvant-based strategy that targets this intrinsic resistance mechanism, destabilizing the ability of Enterobacteriaceae pathogens to respond to GlmS inhibition. This approach therefore unlocks the therapeutic potential of GlmS in clinically relevant pathogens, transforming a previously elusive target into a viable avenue for next-generation antibacterials.

Section Type I&I

Invited Speaker for Symposium 3 – Antimicrobial Resistance June 17, 2026

174 - Using the Gut Microbiome to Contain the Spread of Antimicrobial Resistance

Myles Wheeler[1], Erik Bakkeren[1]

[1]Department of Biological Sciences, University of Calgary

Antimicrobial resistance (AMR) is an increasing global threat, projected to be linked to the deaths of 10 million people globally each year by 2050. AMR is often encoded on DNA plasmids, which can be transferred between compatible bacteria via cell-to-cell contact. This means that plasmid spread is promoted in dense communities where the probability for cell-cell interactions is higher, such as the human gut microbiome.

However, the gut microbiome is also diverse, made up of hundreds of species that must compete for nutrients. Consequently, population sizes of individual species are often restricted, including those that may carry or receive plasmids. In diverse microbial communities, this competition for nutrients can limit the ability of a strain to invade a microbiome, a phenomenon called nutrient blocking. However, the effect of microbial diversity on plasmid spread remains poorly understood. Specifically, it is unclear whether the same competitive forces that limit invasion also reduce the probability that donor and recipient cells meet. To fill this knowledge gap, we explored the impact of a diverse microbiome on the spread of plasmids that carry AMR. Our hypothesis is that nutrient competition within the microbiome restricts plasmid spread by limiting donor growth via nutrient blocking and thereby reducing opportunities for conjugation.

To test this hypothesis, we used a combination of mathematical modelling and experiments. First, we developed a deterministic mathematical model that captures the ecology of plasmid transfer following invasion in the microbiome. Specifically, our model tracks the spread of a plasmid between an initially rare plasmid-carrying donor and a pre-established recipient strain. We find that plasmid spread is boosted when donor-recipient strain pairs differ in their nutrient preferences, as this allows strains to co-occur. However, the addition of the gut microbiome that overlaps in nutrient preferences relative to conjugating strains blocks plasmid transfer.

We then tested our modelling predictions with simple batch culture experiments under anaerobic conditions. using the model species *Escherichia coli*, a common gut microbiome species and a key contributor to the spread of AMR via plasmids. Specifically, we used clinical isolates of *E. coli* carrying extended-spectrum beta lactamase plasmids as donor strains. As recipients, we used *E. coli* isolates from the healthy human gut. As predicted, if an *E. coli* donor strain could not establish itself in the presence of an *E. coli* recipient strain, plasmid transfer was inefficient. However, in cases where the donor and recipient strains could co-occur, plasmid transfer was boosted.

Finally, we repeated the experiment in the presence of 9 human gut microbiota isolates that provide strong nutrient competition against the invading *E. coli* donor strain. We observed that donor invasion and consequent plasmid transfer was reduced compared to when the 9 species were absent. This result validated predictions from the mathematical model and suggest that plasmid spread can be limited via nutrient blocking conferred by a diverse microbial community.

Overall, these findings provide a foundation for designing microbiome-based strategies to limit the dissemination of antimicrobial resistance.

Section Type I&I

Abstract selected for Symposium 3 – Antimicrobial Resistance June 17, 2026

Symposium 4 Abstracts/ Résumés du Symposium 4

13 - Exploring New Chemical Space for Antibiotics with Machine Learning and Bacterial Phenotypic Profiling

Yves Brun[1]

[1]Université de Montréal

Antimicrobial resistance is a major global health threat, yet no antibiotics with a novel mode of action have been approved in the last 20 years. While machine learning (ML) accelerates drug discovery by optimizing molecules in known chemical spaces, it struggles to explore novel spaces where new mechanisms of action might exist. We use predictive and generative active learning ML to explore chemical space for antibiotic discovery. To enhance training, we employ bacterial cell painting, which uses fluorescent dyes to generate detailed phenotypic profiles of compound effects at high throughput. By linking these microscopy profiles to known antibiotics and whole genome CRISPRi depletion data, ML models can infer mechanisms of action. Using high-throughput microscopy screening and iterative active learning loops, we aim to identify and validate new antibiotic candidates in unexplored chemical spaces.

Section Type MGCM

Invited Speaker for Symposium 4 - Microbiology & Artificial Intelligence June 17, 2026

397 - Biocuration, Artificial Intelligence, and the Comprehensive Antibiotic Resistance Database

Andrew McArthur[1]

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Increasing rates of antimicrobial resistance (AMR), i.e., drug-resistant infections (DRI), is a direct threat to modern medicine. In Canada, approximately 26% of infections in 2018 were resistant to the drugs generally used to treat them. Globally, current rates of AMR and predictions for increased rates of DRI are equally dire. Yet, antimicrobial resistance in human populations does not occur in isolation but instead occurs within the broader context of antimicrobial use in agriculture and the pollution of natural environments. Canada has instituted a Federal Action Plan on Antimicrobial Resistance and Use, of which one focus is surveillance of AMR. The Comprehensive Antibiotic Resistance Database (card.mcmaster.ca) is a made-in-Canada database and software platform used around the world for genomic surveillance of antimicrobial resistance genes (ARGs) by applying diverse data science approaches from genomics to protein language models. Yet, with over 13,000 scientific publications on AMR each year and an increasing need for broad interpretive and epidemiological data from the community, CARD is increasing applying either AI-assisted literature triage, natural language processing (NLP) transformers for data extraction, or AI Agents for unsupervised workflows in tandem with expert human biocurators. With the underlying hypothesis that better data will lead to better outcomes, CARD researchers increasingly must span microbiology, biochemistry, computer science, biocuration, and commercialization to support the AMR work in academia, government, and industry.

Section Type: I&I

Invited Speaker for Symposium 4 - Microbiology & Artificial Intelligence June 17, 2026

114 - Machine learning reveals oxidative stress resistance genes across diverse yeasts

Katarina Aranguiz[1], Logan Elkin[2], Laura Delage[3], Sarah Lapolla[3], Kenia Segura-Aba[4], Drew Jordahl[1], Katherine Overmyer[1], Russell Wrobel[1], Joshua Coon[1], Shin-Han Shiu[4], Antonis Rokas[5], Chris Todd Hittinger[1], Linda Horianopoulos[3]

[1]University of Wisconsin-Madison, [2]Medical College of Wisconsin, [3]University of Guelph, [4]Michigan State University, [5]Vanderbilt University

Stress responses are essential for survival under sub-optimal conditions and understanding their mechanisms in yeasts can help us optimize robust growth of useful strains or overcome these responses to treat pathogenic species. Oxidative stress is caused by exposure to reactive oxygen species (ROS), which are highly reactive, oxygen-containing molecules generated during mitochondrial metabolism and encountered during interactions with other organisms as part of their innate immune responses to restrict microbial infection. Therefore, we expected that ROS resistance would be widespread across the yeast phylogeny, however, very little is known about oxidative stress responses in yeasts other than *Saccharomyces cerevisiae* and a few pathogenic species.

To address this, we systematically quantified resistance to the ROS-inducing compound *tert*-butyl hydroperoxide across 284 species in the ancient yeast subphylum Saccharomycotina. We used machine learning-based comparative genomics to build a classification model for ROS resistance and identified gene families whose sizes are predictive of ROS resistance. The most predictive features are enriched in gene families related to cell wall organization and include two reductase gene families. We estimated the quantitative contributions of features to each species' classification using Shapley additive explanations (SHAP). Guided by this analysis, we went on to validate the model's predictions and show that overexpression of the old yellow enzyme (OYE) reductase increases ROS resistance in the otherwise sensitive species, *Kluyveromyces lactis*. Similarly, we generated *S. cerevisiae* mutants lacking multiple mannosyltransferase-encoding genes which rendered them hypersensitive to ROS compared to the parent strain. This work identified gene family expansions of specific enzymes as a previously underappreciated evolutionary mechanism of stress resistance and provided a framework for how machine learning can uncover genetic mechanisms underlying trait variation across diverse species.

Now that we have identified these naturally evolved mechanisms, we are using adaptive laboratory evolution to further probe the mechanisms and constraints of ROS resistance in yeast. Through sequential passaging in increasing concentrations of hydrogen peroxide over ~70 generations we have isolated several independently evolved strains with improved growth in the presence of ROS. By integrating comparative genomics with directed evolution, we are developing a comprehensive understanding of how ROS resistance evolves. Given the importance of ROS in both innate immunity and cellular metabolism, this work has broad implications for antifungal therapeutic development and for optimizing yeast strains used in the bioproduction of lipids and proteins that depend on oxidative metabolism.

Section Type MGCM

Abstract selected for Symposium 4 - Microbiology & Artificial Intelligence June 17, 2026

378 - Postbiotic Supplementation of Sows Shapes Gut Microbiome and Metabolome Development in Neonatal Piglets

Anindita Roy[1], Nahidur Rahman[1], ASM Zisanur Rahman[1], Hooman Derakhshani[1]

[1]University of Manitoba, Department of Animal Science

The early-life gut microbiome plays a fundamental role in regulating intestinal homeostasis, immune maturation, and metabolic programming in neonatal piglets. Vertical microbial transfer from dam to offspring represents a critical seeding event that shapes the trajectory of microbiome succession and long-term gut health. Despite growing interest in nutritional strategies to optimize sow gut health, the downstream impact of maternal postbiotic supplementation on piglet microbiome and metabolome development remains poorly understood. Gilts were enrolled at first conception and assigned to either a control diet or a diet supplemented with a commercial postbiotic product containing *Saccharomyces* yeast fermentation derivatives, vitamins, and antioxidants throughout gestation. Piglets were raised in their respective farrowing pens, and fecal samples were collected at days 3, 7, and 21 (weaning) for gut microbiome profiling by shotgun metagenomic sequencing (Illumina NovaSeq; mean 15.4M reads/sample). Weaning-day samples were additionally subjected to untargeted metabolomics with multivariate and machine learning-based analysis. Microbial richness increased over time in both groups. At weaning, piglets from supplemented sows had higher richness but lower diversity than controls ($p < 0.05$). Age was the dominant driver of compositional change, with a progressive decline in Pseudomonadota and Bacteroidota and a rise in Firmicutes, consistent with normal microbiome maturation. Piglets from supplemented sows showed higher relative abundance of *Prevotella copri*, *Parabacteroides distasonis*, and *Intestinimonas massiliensis*. At day 7, their metagenomes were also enriched in reads mapping to carbohydrate-active enzymes (CAZymes), suggesting an earlier shift toward carbohydrate fermentation capacity. Metabolomic profiling at weaning revealed clear group separation by PLS-DA (R^2=0.767). A random forest model built on the top four discriminating metabolites, including traumatic acid, aspartyl-aspartic acid, γ-glutamylvaline, and leukotriene E4, achieved an ROC AUC of 0.826. Pathway enrichment analysis identified arginine and proline metabolism, glycine/serine/threonine metabolism, and tyrosine metabolism as significantly enriched in piglets from supplemented sows, suggesting upregulated amino acid turnover. Maternal postbiotic supplementation during gestation was associated with subtle but consistent differences in early microbiome succession and metabolic phenotype in offspring. These findings support the sow-piglet axis as a relevant target for microbiome-informed nutritional strategies aimed at improving early succession of microbiome and gut health.

Section Type AEM

Abstract selected for Symposium 4 - Microbiology & Artificial Intelligence June 17, 2026

Symposium 5 Abstracts/ Résumés du Symposium 5

420 - Extremes of time and environment - defining the biomes of the deep biosphere?

Barbara Sherwood Lollar Lollar[1]

[1]University of Toronto

Extreme environments and the discovery of deep subsurface biomes, particularly in environments isolated from the influence of the Sun's energy, provide new models for understanding rock-hosted, chemosynthetic life that may exist on other worlds. Recent revolutions in genomics have sparked exciting studies extending exploration of the deep biosphere to development of novel theories of the origin and evolution of subsurface life, and the emergence of ancestral pathways and metabolisms. A critical gap remains in placing time constraints on these evolutionary hypotheses. New insights are required to move the field beyond the still prevalent assumption that microbial life is synchronous with host rocks/fluid inclusions/sediments - a practice that can lead to significant over-estimates of ecosystem residence time. Some recent studies have recognized that life may be as young as the youngest fluids that later permeated or mixed within these systems, or have used short-lived tracers such as 14C to constrain subseafloor fluid histories; demonstrating that overall a quantitative understanding integrating multiple lines of evidence is critical, particularly given the necessity for evolutionary studies to interrogate both marine-hosted systems (necessarily < 200 Ma old) and the wealth of emerging data from continental subsurface systems wherein a longer record of the Earth's more than 4 billion year history may be preserved. Biological evidence alone is insufficient to determine the indigenicity of subsurface life, as extant life, and signs of past life, may have entered the subsurface after the rocks formed, particularly if the host formation has been permeated by younger groundwaters entering the system. "Follow the rock, and the water" is a critical strategy both for investigations of life on Earth, and for astrobiology. Exploration of the origin and evolution of life requires integrated contextual understanding of the rock record and the hydrogeologic cycle through deep time, to constrain timing for the colonization of life, and timescales of possible preservation of extant life, and biomarkers of past life.

Section Type AEM

Invited Speaker Symposium 5 – Extremophiles June 17, 2026

104 - Origin and Adaptation of Alkaliphilic Bacteria in Deep Geological Time

Lianchun Yi[1], Varada Khot[2], Jason de Koning[1], Marc Strous[1]

[1]University of Calgary, [2]Friedrich Schiller University Jena

Alkaline soda lakes are a rich source of bacteria and enzymes for industrial applications. More recently, they have gained attention as a possible environment that spawned life on early Earth. Using ancestral reconstruction and stochastic character mapping, we explore the evolutionary origins of current soda lake bacteria. When did they first evolve? What did the Earth look like during that time? How hard is it to adapt to soda lake environments? We show that transition into alkaline soda lakes is supported by thousands of genes, each contributing small fitness gains. However, acquisition of two specific transporters explains more than half of transitions into alkaline soda lakes. We show that these transporters largely evolved in the context of extant alkaliphilic lineages. The presence of protein domains associated with these transporters in the last universal common ancestor is consistent with the possible emergence of life from an alkaline soda environment.

Section Type AEM

Invited Speaker Symposium 5 – Extremophiles June 17, 2026

155 - The IME1s: A Novel Lineage of (likely Filamentous) Viruses in *Dehalococcoides*

Camilla Nesbo[1, 2], Elizabeth Edwards[1]

[1]University of Toronto, [2]University of Alberta

Dehalococcoides mccartyi are small but mighty - these specialized anaerobic bacteria play a key role in detoxifying chlorinated pollutants in contaminated groundwater and sediments. Despite their compact genomes, they host a surprisingly diverse array of mobile genetic elements. Here, we analyse in detail one such element: the integrative and mobilizable element 1, or IME1.

IME1s are the most abundant elements in metaviromes from KB-1, a well-characterized *Dehalococcoides*-containing mixed culture, when it is grown under conditions optimal for dichlorination. We characterized 20 IME1s across various *D. mccartyi* genomes and one from *Dehalogenimonas alkenigignens*. Ranging from ~21 to ~28 kb, these elements occur both as chromosomal integrations and as circular double-stranded replicons.

Bioinformatic analysis revealed a highly conserved genomic architecture, with 14 hierarchical orthologous groups (HOGs) shared across all 21 IME1s. None of these proteins resemble hallmark proteins of tailed bacterioviruses. Instead, multiple lines of evidence point to filamentous viruses. One conserved HOG shares structural and sequence similarity with the Zot/pI-like ATPase (the sole marker protein for filamentous bacterioviruses) and they encode several small proteins with predicted transmembrane domains and signal peptides, both hallmarks of filamentous bacterioviruses. They are likely to be constitutively expressed without killing the host found at high abundance in both metagenomes (~ 7X the genome coverage) and metaviromes (up to 1000X the genome coverage) when *D. mccartyi* is abundant. Electron micrographs of viral fractions from KB-1 and other *Dehalococcoides* cultures consistently reveal filamentous structures.

Taken together, we propose that IME1s represent a novel lineage of likely double-stranded filamentous viruses. Database searches further suggest their presence across the Chloroflexota phylum, though they appear to be restricted to it. These findings open new questions about virus diversity in anaerobic, pollutant-degrading microbial communities.

Section Type AEM

Abstract selected for Symposium 5 – Extremophiles June 17, 2026

303 - Propagating effects of permafrost thaw slumps on microbial communities throughout Arctic stream networks in the Northwest Territories

Marina Taskovic[1], Brian Lanoil[1], Jeremie Mahaux[1], Suzanne Tank[1]

[1]Department of Biological Sciences, University of Alberta, Edmonton AB, Canada

Permafrost thaw in northwestern Canada has accelerated in recent decades due to rapid warming and increased precipitation. The degradation of ice-rich permafrost transforms landscapes, triggering widespread mass-wasting features known as thaw slumps. Across the Peel Plateau in the Northwest Territories, these thaw slumps mobilize substantial volumes of materials rich in organic matter, nutrients, and other solutes. The lateral transport and deposition of thawed materials into streams shift hydrological, sedimentary, and geochemical regimes, with cascading effects on biogeochemical cycles, food webs, and water quality. Central to these processes are prokaryotic microbial communities, which drive the transformation of elements that shape ecosystem function and services. However, the responses of microbial communities within slump-affected stream networks remain poorly understood.

We conducted a two-year (2022-2023) catchment-scale investigation in the 1,100 km^2 Stony Creek watershed on the Peel Plateau. Our study tracked microbial community responses along the slump-stream continuum, including rill water runoff from seven thaw slumps, impacted headwaters, major tributaries, and a transect along the Stony Creek mainstem. We characterized microbial communities using 16S rRNA amplicon sequencing and combined these data with stream physicochemical properties, dissolved (DOC) and particulate organic carbon (POC) composition, and landscape metrics to identify drivers of microbial community response. We also assessed microbial functional potential and biomass production using marker gene-based functional predictions (FAPROTAX) and tritiated leucine incorporation experiments.

Community divergence downstream of rill water inflow covaried strongly with changes in sediment loading and associated particulate organic matter. Among headwaters, increased slump influence led to a shift in microbial community composition toward a higher abundance of taxa functionally associated with anoxic conditions and a reduced, low-energy carbon pool, reflecting the transition to a system dominated by POC. Patterns along the Stony Creek mainstem support these trends: sediment influx from tributaries not only drove spikes in community dissimilarity but also showed that such effects persisted and propagated along the river continuum. Notably, despite the predominance of POC, bacterial biomass production remained strongly correlated with DOC throughout the mainstem, even though DOC comprised only a small fraction of the total carbon pool relative to POC.

Through this work, we aim to document how microbial communities transform as thaw slump materials are transported through fluvial networks. Establishing these patterns is critical for understanding biogeochemical cycling in thermokarst-affected landscapes, where terrestrial-aquatic connectivity is pronounced. Collectively, our findings offer a baseline for microbial community dynamics in this region, complementing other interdisciplinary research by integrating microbial ecology with physical and chemical drivers of change. As climate change and permafrost thaw intensify in Arctic ecosystems, understanding the role of microbial communities becomes increasingly important. By characterizing stream microbial diversity, predicting functional profiles, and quantifying microbial activity, we advance our understanding of the mechanisms driving biogeochemical changes and their broader impacts on food webs, water quality, and greenhouse gas exchange.

Section Type AEM

Abstract selected for Symposium 5 – Extremophiles June 17, 2026

Symposium 6 Abstracts/ Résumés du Symposium 6

408 - Building climate-resilient food systems through soil microbiome stewardship

Mallory Choudoir[1]

[1]North Carolina State University

Climate change, land use intensification, and other anthropogenic activities threaten to disrupt agricultural soil microbiomes and the vital ecosystem functions they confer. Engaging in microbiome stewardship practices that maintain soil microbial biodiversity and function can help build climate-resilient food systems. This session will demonstrate the principles of microbial stewardship with two case studies spanning production scales that highlight the challenges and opportunities of soil microbiome stewardship. The first project involves community engaged research and heirloom collard cultivation, and the second project investigates soil microbiome dynamics and row crop productivity in salt-impacted habitats. These projects highlight unique challenges for practicing microbiome stewardship and offer potential strategies for building more climate resilient food systems.

Section Type AEM

Invited Speaker Symposium 6 – Microbiology & Society June 17, 2026

386 - From theory to practice of microbiome stewardship

Kieran O'Doherty[1]

[1]University of Guelph

Since the advent of microbiome science, evidence has been accumulating to support our understanding that:

1) Microbiomes are essential to support healthy functioning of humans, other animals, plants, and larger ecosystems.

2) Microbial ecosystems are changing as a result of human activities (e.g., widespread use of biocidal chemicals; environmental microplastics; pharmaceutical runoff; industrial pollutants).

3) These changes are associated with adverse consequences for hosts and ecosystems (e.g., for humans, asthma, IBD, neuroinflammatory conditions).

This knowledge provides an important foundation for actions to support the healthy functioning of humans and other organisms, and many microbiome scientists have successfully developed interventions to improve microbiome health of humans and other hosts. However, very little has been done to address degradation of microbiomes on a societal level.

In this presentation, I argue that the effects of degrading microbiomes need to be understood as collective societal problems. By corollary, efforts to address these problems need to occur at the level of public policy, institutional practices, and international agreements. While also necessary, efforts toward improving health at smaller scales are insufficient to address the root causes of these problems. For example, developing new therapies for IBD are necessary, but will not address the environmental and systemic factors that are leading to rising prevalence of IBD in the first place.

Existing initiatives, such as One Health, microbial biobanking, and planetary health have made important progress in this regard but also have their limitations. For example, with few exceptions, One Health initiatives relating to microbes tend to focus on combating pathogens rather than maintaining diversity of microbial ecosystems that are essential for host functioning. The concept of microbiome stewardship complements existing initiatives by providing a framework for taking action to safeguard microbiomes. At minimum, microbiome stewardship involves:

1) Identifying the (human) communities, organisms, or environments being threatened by microbiome changes.

2) Identifying the ways in which microbiomes are involved in maintaining host or ecosystem health in those specific contexts.

3) Identifying factors involved in changing microbiomes in ways leading to deteriorating host or ecosystem health.

4) Identifying social, regulatory, or institutional mechanisms for limiting or eliminating negative effects on microbiomes in specific contexts.

I discuss what has been accomplished to date to develop the concept of microbiome stewardship. I also discuss current work on developing metrics to measure and implementing stewardship in practice, and initial steps toward integrating knowledge of microbiomes and attention to their preservation into policy frameworks.

I conclude by outlining next steps in the development of microbiome stewardship and an invitation to participate in the important work of safeguarding microbial ecosystems that are foundational for life on Earth.

Section Type AEM

Invited Speaker Symposium 6 – Microbiology & Society June 17, 2026

336 - iMicroSeq: Integrative resources supporting clinical and environmental microbial sequence data

Nima Tehrani[1], Baofeng Jia[1], Justin Richardsson[2], Charlie Barclay[1], Leonardo Rivera[2], Edmund Su[2], Johanie Gilbert[1], Carolina Koutras[3], Mohamed Kharbouch[3], Bhavik Bhagat[2], Robert Delatolla[4], Casey Hubert[5], Michael Parkins[5], William Hsiao[1], Hector Galvez[6, 7], Guillaume Bourque[6, 7], Math'ieya Alatini[3], Erin Gill[1], iMicroSeq Leadership Table[8], Melanie Courtot[2], Emma Griffiths[1], Fiona Brinkman[1]

[1]Simon Fraser University, [2]Ontario Institute for Cancer Research, [3]One Yukon, [4]University of Ottawa, [5]University of Calgary, [6]McGill University, [7]Canadian Centre for Computational Genomics, [8]iMicroSeq

Environmental DNA and RNA sequence-based monitoring (collectively "eDNA") is a revolutionary technology that can provide a more wholistic view of microbial/viral prevalence. Wastewater-based surveillance (WBS) with eDNA can complement more limited, biased case-based clinical testing for pathogens and antimicrobial resistance prevalence, allowing cost-effective and inclusive monitoring of whole communities. eDNA-based analyses also aid monitoring supporting agriculture, aquaculture, mining, defence, and environmental health. However, these monitoring efforts are currently scattered, as different regions and scientific disciplines often work separately, leading to gaps in our understanding. This fragmentation makes it harder to identify trends in diseases and environmental changes, which often require One Health integrative approaches (human, other animal, and environmental data integration).

iMicroSeq.ca (inclusive, integrative Microbial Sequencing) is a national initiative grounded in the principle of complementing clinical microbial sequence data with environmental DNA/RNA water-based analysis to wholistically Learn from Water to improve the health of people, animals, and the environment.

Here we describe interlinked resources, including the iMicroSeq Data Portal for sequence and qPCR data, and tools to support modelling and visualization of the data. Our work is rooted in a data governance framework that supports FAIR Principles, data security, privacy, recognition of submitters, robust data standards/metadata specifications, government-academic collaboration, and data governance transparency. This fully Canadian sovereign resource is an extension of the VirusSeq Data Portal, originally developed to host Canadian clinical SARS-CoV-2 sequences. In addition to the clinical data, the iMicroSeq data portal now also accommodates a range of environmental sample data types, including wastewater and other water qPCR and metagenomics data, for any microbial species. Contextual data (i.e., metadata) from samples from across the country are curated and harmonized through ontology-based data specifications, including those of the Public Health Alliance for Genomic Epidemiology, ensuring consistency, interoperability, and reusability. Metadata include sampling context (e.g., geography, geolocation, precipitation, timing), sampling protocols and processing methods, sequencing or PCR quantification assay types, and contact information including for sample collectors and data stewards. In addition, the associated ChùNet.ca project is supporting development of an Indigenous-led local knowledge and data sharing network for monitoring that reflects community preferences.

This iMicroSeq resource is being used to perform a demonstrative integrative analysis of SARS-CoV-2 and Influenza A waves from across the country. This work links urban WBS data at the postal code level with over 500 geospatial and socio-environmental data measurements, obtained from the Canadian Urban Environmental Health Research Consortium. The objective of this analysis aims to more integratively and agnostically identify environmental or social factors associated with onset and persistence of COVID-19 or Influenza waves.

Collectively, we describe an inclusive framework that aims to enable robust microbial/eDNA sequence data linkage, discovery, and analysis, with relevant environmental/other data to advance equitable water-based monitoring of pathogens and other microbes within a One Health framework. Results of this work supports identifying predictors of disease trends, improving forecasting of community-level risk, and informing public health policy and interventions.

Section Type AEM

Abstract selected for Symposium 6 – Microbiology & Society June 17, 2026

226 - Microbiological considerations for implementation of a deep geological repository for used nuclear fuel storage

Rachel Beaver[1], Katja Engel[1], Emilie Spasov[1], Anu Chadha[1], Huan Tong[2], Harmanpreet Sidhu[3], Claire Tully[4], Jessie McDonald[4], W. Jeffrey Binns[5], Chang Seok Kim[5], Peter Keech[5], James Noël[4], Greg Slater[3], Myrna Simpson[2], Josh Neufeld[1]

[1]University of Waterloo, [2]University of Toronto, [3]McMaster University, [4]Western University, [5]Nuclear Waste Management Organization

Deep geological repositories (DGRs) are the globally accepted solution for the safe, long-term storage of used nuclear fuel, and rely on multiple engineered barriers to isolate waste deep underground. To ensure the long-term integrity of these systems, it is critical to characterize microorganisms associated with DGR design components, as microbial activities could compromise engineered barriers over the millennia for which a DGR must remain functional. Of particular importance are sulfate-reducing bacteria (SRB), which produce sulfide—the only compound capable of corroding the copper coating of used fuel containers (UFCs) under expected DGR conditions once oxygen is depleted. In Canada's DGR design, bentonite will surround copper-coated UFCs to inhibit microbial growth through swelling upon contact with water, which elevates pressure and reduces pore space and water activity. Accordingly, a primary goal of DGR design is to identify the minimum bentonite dry density necessary for suppression of microbial growth. We investigated microbial communities in bentonite exposed to a range of DGR-relevant conditions. Oxic pressure vessel experiments demonstrated that microbial growth occurs during initial bentonite saturation, but is suppressed post-saturation with a minimum bentonite dry density of 1.40 g/cm^3. Similar trends were observed under anoxic conditions, with additional minimal SRB growth detected at bentonite-vessel interfaces. To simulate *in situ* pressures, bentonite-containing modules were deployed to the seafloor and showed a similar absence of microbial growth. Finally, modules incubated in boreholes 300 m below ground at the planned Canadian DGR host site revealed minor interface-associated microbial growth, but no growth within inner bentonite layers, nor of SRB in any layer despite their presence in the surrounding subsurface water. Overall, our results support the use of bentonite at a dry density of at least 1.40 g/cm^3 as an effective solution for limiting microbial activity in saturated DGR environments.

Section Type: AEM

Abstract selected for Symposium 6 – Microbiology & Society June 17, 2026

196 - Structure-guided discovery of novel antiphage defence glycosylases

Landon Getz[1], Amy Qian[1], Y. Vivian Liu[1], Sam Fairburn[1], Mahnoor Butt[1], Yan-Jiun Lee[2], Peter Weigele[2], Karen Maxwell[1]

[1]Department of Biochemistry, University of Toronto, [2]New England Biolabs

Bacteria and their cognate viruses (phages) are locked in a billion-year-old evolutionary arms race. Bacteria have evolved robust and diverse mechanisms to defend themselves against phage infection, and phages have developed equally elegant counter-strategies. As multi-drug resistant pathogens threaten our ability to treat bacterial infections at a global scale, using phages to treat these infections offers a solution. However, the same bacterial immune systems shaping this conflict will be part of deciding whether a therapeutic phage succeeds in a patient. Understanding bacterial antiphage immunity is therefore fundamental to deploying phage therapy and has become one of the fastest-moving areas of bacterial biology.

Here, we have identified two unannotated proteins with predicted DNA glycosylase folds, Dag1 and Dag2, that robustly defend against restriction-resistant phages carrying non-canonical guanosine bases such as 7-deazaguanine. Importantly, these are the first functional guanosine-specific DNA glycosylases to be described in antiphage defence. Both of these enzymes were identified in bacterial integrons, large genomic repositories of adaptive gene functions, that ourselves and others have recently shown to be hot spots for antiviral immunity. These elements capture gene cassettes from the environment to build reconfigurable libraries of adaptive functions, and can grow to over 200kb (~5% of the genome) in length. Biochemically, we have shown that Dag1 and Dag2 function as bifunctional glycosylase/AP lyases, excising the modified guanosine base and cleaving the phosphodiester backbone to generate single-stranded nicks in DNA. Cumulative nicks across phage genomes, as shown by *in vitro* incubation of Dag1 with guanosine-modified phage DNA, lead to DNA degradation and the termination of phage replication.

To better understand the breadth of antiviral glycosylases in bacterial immunity, we built a discovery pipeline pairing protein structural prediction with targeted structural searches. Importantly, this pipeline does not rely on sequence similarity, which is unable to identify highly-divergent families of DNA glycosylases. Treating integrons as antiviral hot spots, we assembled a structural library of 76,370 proteins from 907 integrons across 1,094 non-redundant *Vibrionaceae* genomes. Searching with Dag1/2 as structural bait recovered 109 unannotated glycosylase-like proteins, clustering into 24 highly divergent families (<30% sequence identity, >80% pairwise coverage) that nonetheless retain conserved active-site architecture. Seventeen of these 24 families are functional *in vivo,* reducing or eliminating infection by guanosine-modified phages. These glycosylases co-occur with mobile genetic elements and are distributed across bacterial phyla, suggesting that enzymatic excision of modified guanosines is a widespread antiviral strategy. Finally, we show that this approach is generalizable by searching for structural homologues of Brig1, an antiphage DNA glycosylase that targets modified cytosine residues. Using this approach we identified a novel subtype of Brig1, called Brig2, that targets modified thymidine residues in phage DNA.

Taken together, we have uncovered a remarkably widespread mechanism that bacteria use to protect themselves from infection by phages that are typically resistant to DNA-targeting antiviral immunity. These findings expand the known repertoire of antiphage immunity and provide a rational basis for engineering therapeutic phages that can evade glycosylase-mediated defence.

Section Type MGCM

Abstract selected for Symposium 6 – Microbiology & Society June 17, 2026

Symposium 7 Abstracts/ Résumés du Symposium 7

409 - A conserved role for a deubiquitinase in phage tail assembly

Alan Davidson[1], Bianca Garcia[1], Yurima Hidalgo-Reyes[1]

[1]University of Toronto

Phage tails are complicated, multifunctional nanomachines. They mediate specific attachment of phage particles to the cell surface, penetration of the cell envelope, and delivery of the genome to the cell interior. Non-contractile tails, such as those of *E. coli* phage lambda, are composed of many copies of 7 to 10 different proteins that polymerize into a tube more than 100 nm in length. Successful assembly of this intricate structure occurs through multiple defined and regulated steps, many of which remain mysterious. This talk will describe a newly discovered role for a conserved family of deubiquitinases that is required for tail assembly of phage lambda and a large number of related phages.

Section Type MGCM

Invited Speaker Symposium 7 - Bacteriophage and Bacterial Defense Systems June 18, 2026

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406 - Capturing dynamic phage-pathogen coevolution by clinical surveillance

Kimberley Seed[1]

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Bacteria harness diverse defense systems that protect against phage predation, many of which are encoded on horizontally transmitted mobile genetic elements (MGEs). In turn, phages evolve counter-defenses, driving a dynamic arms race that remains underexplored in human disease contexts. For the diarrheal pathogen *Vibrio cholerae*, a higher burden of its lytic phage, ICP1, in patient stool correlates with reduced disease severity. However, direct molecular evidence of lytic phages driving selection of epidemic *V. cholerae* has not been demonstrated. Here, through clinical surveillance in cholera-endemic Bangladesh, we capture the acquisition of a parasitic anti-phage MGE, PLE11, that initiated a selective sweep coinciding with the largest cholera outbreak in recent records. PLE11 exhibited potent anti-phage activity against co-circulating ICP1, explaining its rapid and dominating emergence. We identify PLE11-encoded Rta as the novel defense responsible and provide evidence that Rta restricts phage tail assembly. Using experimental evolution, we predict phage counteradaptations against PLE11 and document the eventual emergence and selection of clinical ICP1 that achieve a convergent evolutionary outcome. Finally, we discover how PLEs balance their dependence on ICP1 tail proteins for horizontal transmission with the restriction of phage tail assembly by Rta: PLEs construct chimeric tails comprised of both MGE- and phage-encoded proteins to ensure their transmission. Collectively, our findings reveal the molecular basis of the natural selection of a globally significant pathogen and its virus in a clinically relevant context.

Section Type I&I

Invited Speaker Symposium 7 - Bacteriophage & Bacterial Defense Systems June 18, 2026

417 - From the Prairies to the Planet: Phage Therapy and the Global Fight Against Superbugs

Greg German[1, 2, 3]

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Antimicrobial resistance is driving renewed interest in bacteriophage therapy as a potential adjunct or alternative to conventional antibiotics. This presentation will provide a concise overview of modern phage therapy from a Canadian clinical microbiology perspective, highlighting current applications in multidrug-resistant and chronic infections. Key topics will include phage susceptibility testing, biofilm-associated disease, translational challenges, and evolving regulatory considerations. The session will emphasize both the opportunities and limitations of integrating phage therapy into contemporary microbiology and infectious diseases practice, while examining how Canadian research and clinical collaborations contribute to the broader global response to antimicrobial resistance.

Section Type I&I

Invited Speaker Symposium 7 - Bacteriophage & Bacterial Defense Systems June 18, 2026

17 - Tail fibre diversification enables phage recognition of diverse type IV pili

Ikram Qaderi[1], Hanjeong Harvey[1], Yao Shen[2], Ylan Nguyen[1], Amogelang Raphenya[1], Isabelle Chan[1], Alba Guarné[3], Andrew McArthur[1], Lori Burrows[1]

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Bacterial surface receptors evolve rapidly under selective pressures such as immune recognition and viral predation, creating a fundamental challenge for bacteriophages (phages) that rely on these receptors for infection. How phages maintain infectivity despite receptor diversification remains poorly understood. Type IV pili provide a tractable system to address this problem, as they serve as receptors for diverse phages while exhibiting extensive sequence and chemical variation.

Type IV pili are dynamic, retractile filaments assembled from thousands of pilin subunits and are broadly distributed in both archaea and bacteria. In *Pseudomonas aeruginosa*, the major pilin PilA is highly diverse across strains but retains a conserved structural scaffold required for pilus assembly. This diversity includes variation in sequence and post-translational glycosylation, which alters susceptibility to some phages. However, how pilus-dependent phages recognize receptors that vary substantially in sequence and surface chemistry remains unclear. Here, we tested whether phages differ in their tolerance to pilin diversification and whether this variation is associated with tail fibre architecture.

Analysis of PilA sequences from more than 1,300 nonredundant *P. aeruginosa* genomes identified 53 distinct pilin variants corresponding to 319 unique pilin-sequence type combinations. Mapping sequence conservation onto structural models showed that variation is concentrated at solvent-exposed residues, while the structural core remains conserved, indicating that diversification alters surface chemistry without disrupting pilus assembly.

To determine how this diversity influences phage recognition, representative pilins were expressed in a common genetic background. Several pilus-dependent phages infected strains expressing divergent pilins, demonstrating tolerance to receptor variation. To define the basis of this tolerance, we compared two phages with contrasting phenotypes: JBD26, which is sensitive to pilin glycosylation, and DMS3, which retains infectivity. A chimeric JBD26 phage carrying DMS3 distal tail fibres restored infectivity on glycosylated pili, demonstrating that receptor recognition is determined by these proteins.

Charge-swap substitutions at solvent-exposed residues strongly reduced JBD26 infectivity but had minimal effects on DMS3, indicating that JBD26 depends on the electrostatic topology of the pilus surface, whereas DMS3 tolerates broader receptor variation. Structural analysis revealed two distinct classes of tail fibres associated with these phenotypes: JBD26-like fibres with conserved receptor-binding domains and DMS3-like fibres with structurally diversified and variable binding regions. Consistent with these differences, phages encoding DMS3-like fibres tolerated electrostatic substitutions and pilin glycosylation, while JBD26-like fibres were sensitive to receptor perturbations.

Together, these findings suggest that modular diversification of receptor-binding proteins provides a structural route by which phages accommodate receptor evolution. More broadly, these principles can inform the selection or engineering of broad-host range phages for therapeutic applications.

Section Type MGCM

Abstract selected for Symposium 7 - Bacteriophage & Bacterial Defense Systems June 18, 2026

Symposium 8 Abstracts/ Résumés du Symposium 8

419 - Tackling legacy methane emissions: methane oxidation on and within municipal landfills

Laura Hug[1]

[1]University of Waterloo

Methane emissions from municipal solid waste landfills contribute to ~10% of global methane emissions. Canonically, the main microbial contribution to methane control is from methanotrophic bacteria present in landfill cover soils (LCS). Surface methane emissions from older capped landfills are frequently considered “legacy methane” in emission reduction budgets, with limited expectation for mitigation. Improvements to methanotroph activities and the potential for anaerobic methane oxidation within the buried waste are two mechanisms to reduce this “legacy methane” and adjust the baseline emissions expected for Canada’s aging landfills. We have conducted two surveys exploring the methane dynamics at older, capped landfills. First, we explored the impact of hot-spots in cover soil emissions: highly emissive localized sites, whose activity and location can vary over daily, seasonal, and multi-year timescales. We surveyed microbial community composition, methane flux, and soil chemistry across a decommissioned capped landfill cover over a total period of 8 years. We had the opportunity to observe methanotroph dynamics under variable methane loads, due to alterations to the landfill gas capture system that diminished methane emissions in one region, but also developed two new hot spots in a different area of the landfill. Active hot spots were lower in total nitrogen relative to other sites, indicating that methanotrophy was likely N-limited. Methane flux was significantly correlated with shifts in community composition, as were soil moisture content, pH, dissolved organic carbon, Ca^{2+}, K^{+}, Na^{+}, and Cl^{-}. Hot spots were dominated by methane oxidizing bacteria belonging to genera *Methylobacter*, *Crenothrix*, and *Methylomicrobium*, whereas sites without exposure to methane had much more diverse communities with methanotrophs constituting <1% of the community. Former hot spots saw an overall decline in total methanotroph relative abundance, but still maintained a higher relative abundance of methanotrophs than sites that had never been hot spots. Additionally, methane flux at the former hot spots was significantly more negative than at completely inactive sites, indicating increased oxidation of near-atmospheric concentrations of methane. Secondly, we identified the genomic potential for anaerobic methane oxidation in older waste. In a metagenome-based examination of a landfill in the North Eastern U.S., older landfill cells contained unexpected diversity of predicted anaerobic methane oxidizers, including members of the *Methanoperedens*. Taken together, this work explores microbial avenues for reducing methane emissions from older landfills, whose modeled future methane emissions are currently treated as immutable.

Section Type AEM

Invited Speaker Symposium 8 – Microbial Ecology & Sustainability June 18, 2026

418 - Sustainability in Canada's Oil and Gas Industry - How Can Microbes Help?

Lisa Gieg[1]

[1]University of Calgary

Global energy needs are still largely met by combusting hydrocarbon-based fuels despite an increase in efforts to develop more renewable energy technologies. Given this continued reliance on oil and gas, along with the known contributions of this and other industries to negative climate change effects, it is imperative to ensure that such fossil energy-related operations are environmentally sustainable. Microorganisms are ubiquitous throughout oil and gas operations and as such can feasibly contribute to sustainability in this industry. For example, many microorganisms can utilize hydrocarbons as carbon and energy sources, which can contribute to the bioremediation of any spilled fuels into environment and to sustained energy production from reservoirs, both of which are positive effects. Microbial activities can also contribute to many negative effects such as the souring of subsurface reservoirs and the corrosion of metal infrastructure. This talk will highlight some examples of how a better understanding of microbial communities and activities can assist with beneficial applications related to hydrocarbon biodegradation and towards early detection or prevention of detrimental effects such as souring and infrastructure corrosion that can lead to pipeline spills. Working towards an improved understanding of the microbial ecology associated with various aspects of Canada's fossil energy industry can help to ensure ongoing sustainable energy recovery operations with minimal environmental impact.

Section Type AEM

Invited Speaker Symposium 8 – Microbial Ecology & Sustainability June 18, 2026

266 - Decoding Microeukaryote Microbiome Interactions: Implications for Marine Ecosystems and Biotechnological Potential

Alexander Mora Collazos[1], Claudio Slamovits[1]

[1]Dalhousie University

Microorganisms play a fundamental role in maintaining ecosystem balance and regulating key processes on Earth, including energy flow, biogeochemical cycles, and climate dynamics. Despite their importance, environmental microorganisms and the complex networks they form remain poorly understood. This work seeks to uncover and unravel the complex network of interactions between microeukaryotes and their microbiome, revealing how these relationships drive ecological dynamics and unlock biotechnological potential. This project explores the bacterial microbiome associated with the heterotrophic dinoflagellate *Oxyrrhis marina.* To address who makes up these microbiomes and how these associations function, we used a multidisciplinary and multi-omics approach. Microbiome and metagenomic analyses were employed to identify microbiome composition, while classical microbiology techniques combined with whole-genome sequencing enabled in-depth characterization of culturable microorganisms. Microbial interactions were investigated using chemotaxis assays, co-culture experiments, metabolic network predictions, and differential gene expression analyses based on RNA sequencing. Results revealed that the microbiome of *O. marina* comprises more than 100 bacterial species. Behaviorally, *O. marina* exhibited both positive and negative chemotactic responses depending on the bacterial partner. Its growth rate also varied in co-culture, in some cases being negatively affected, suggesting not only mutual dependence but also a finely balanced microbiome structure and composition. Metabolic predictions and gene expression analyses point to complex interactions among organisms driven by complementary metabolic pathways. Additionally, genomic analysis of culturable bacteria revealed a wide range of potential applications, including strains capable of producing antifungal compounds that demonstrated effectiveness against phytopathogenic fungi in laboratory assays. Among the microorganisms associated with the microbiome, bacteria capable of degrading hydrocarbons and phthalates were identified. Notably, one bacterial strain exhibited high efficiency in the reduction of heavy metals such as hexavalent chromium, achieving a reduction rate of up to 18 ppm per day at concentrations of 400 ppm. Furthermore, bacteria capable of biomineralization processes and the synthesis of essential cofactors and vitamins, such as cobalamin, were identified. This occurs through a relatively understudied mechanism involving both chromosomal and plasmid-encoded genes. The findings from this project suggest that microbial associations and interdependence in marine environments are more complex than previously recognized, indicating that classical models of symbiotic relationships are insufficient to fully explain these dynamics. Moreover, marine microeukaryotes play a key role as reservoirs of rich microbial diversity with significant biotechnological potential. They may also contribute critically to the modulation, transformation, and resilience of marine ecosystems.

Section Type: AEM

Abstract selected for Symposium 8 – Microbial Ecology & Sustainability June 18, 2026

146 - Understanding the impact of thaw-driven nutrient influx on microbial carbon cycling in the Mackenzie River Basin

Laura Schnell[1], Jordyn Broadbent[2], Jordan Musetta-Lambert[2], Thomas Reid[3], Renee McFadyen[3], Jonathan Keating[2], Andrew Cameron[1]

[1]University of Regina, Regina, Saskatchewan, Canada, [2]Environment and Climate Change Canada, Saskatoon, Saskatchewan, Canada, [3]Environment and Climate Change Canada, Burlington, Ontario, Canada

Thaw-slumps release large quantities of previously stored nutrients into Arctic watersheds, drastically altering their biogeochemistry and hydrology. However, the effects of this nutrient influx on microbial communities and their role in carbon cycling remain largely uncharacterized. To address this, we investigated how sediment input from thaw slumps in the Mackenzie River Basin affect microbial carbon cycling using genomics approaches. We collected sediments from upstream (reference) and downstream (slump-impacted) sites and incubated them for one month under simulated low-flow river conditions using a 2-meter annular flume. During incubation, we conducted decomposition experiments using leaf packs and cotton strips as standardized substrates for microbial breakdown. Weekly sampling allowed us to track substrate decomposition and shifts in microbial community structure and function using metagenomics. We found clear differences in microbial composition and functional potential between upstream and downstream incubations for both carbon substrates, as well as increased leaf decomposition, more reduced nutrient conditions, and differing dissolved carbon profiles in downstream incubations, indicating distinct microbial metabolic processes. This work provides a proof-of-concept for investigating the impact of thaw slump sediment inputs on microbial carbon cycling in Arctic rivers. These insights into the microbial functional response at the mesocosm level have informed the collection of *in-situ* sediment and water samples from thaw-slumps in the Peel Plateau, NWT. These samples have been profiled for metagenomics, metatranscriptomics, greenhouse gas characterization, and targeted metaproteomics to reveal patterns of microbial carbon cycling in response to thaw slump activity. Here we also present metagenomic insights into the *in-situ* activity of Arctic aquatic microbial communities, which reveal both a core functional response, and insight into the diversity of taxonomic and functional capacity of these communities.

Section Type AEM

Invited Speaker Symposium 8 – Microbial Ecology & Sustainability June 18, 2026

100 - Characterization of Unprecedented Aerobic Anoxygenic Phototroph Isolated from Saline Springs with Rhizobium prophage.

Etse Oyoto[1], Katia Messner[1], John Kyndt[2], Marike Palmer[1], Vladimir Yurkov[1]

[1]University of Manitoba, [2]Bellevue University

Aerobic anoxygenic phototrophs (AAP) perform photosynthesis using the pigment bacteriochlorophyll *a* (BChl *a*). They are an intriguing group of bacteria, because while no oxygen is produced during this process, the photic cyclic pathway can only be performed under aerobic conditions. This distinguishes them from other anoxygenic phototrophs, which require anoxic conditions. Light gives AAP an advantage over other heterotrophs, acting as an additional source of energy and is especially beneficial in nutrient poor environments. AAP notably lack RuBisCo, are strictly aerobic, and unable to grow under autotrophic conditions. Their photosynthetic apparatus includes BChl *a* incorporated in a reaction center (RC) and supported by one or more light-harvesting complexes.

A yellow-pigmented strain MS438, in the *Aurantimonadaceae* family, was isolated from saline springs close to Lake Winnipegosis, Manitoba, Canada. Sequencing of the 16S rRNA gene showed 99.15 % relatedness to *Fulvimarina uroteuthidis* strain 2208YS6-2-32^T and 97.45% and 96.59% similarity to *Fulvimarina endophytica* strain 85^T *Fulvimarina fulva* strain MAC8^T, respectively. While MS438 was cultured from an inland flowing saline spring far from continental shores, its genus relatives were isolated from different parts of the ocean like seawater, hydrothermal vents, and in one instance, a squid. This alphaproteobacterium strain is an AAP as it is strictly aerobic, is unable to grow under autotrophic conditions, and produces BChl *a* incorporated into the RC and supported by a light-harvesting complex I. Further genomic evidence of MS438 shows a photosynthetic gene cluster with all the required genes for anoxygenic photosynthesis. Previously, four species in the *Fulvimarina* genus had no BChl detected, and the remaining two were not screened for this pigment. Thorough physiological testing shows MS438 to be halotolerant (0-12% NaCl), psychotolerant (4-32ºC) and slightly alkalitolerant (pH 5.5-9).

Genome analysis presented fascinating insights, such as the unusual integration of a possible *Rhizobium* phage. It is closely related to a prophage that was originally isolated from *Rhizobium etli* in bean plants' rhizosphere of crop fields located in México. This is intriguing, due to not only the large geographical distance, but also the polarity in climates and type of isolation environment. Analysis shows MS438 genome similarity falls below the cutoffs for average nucleotide identity (> 95%) and digital DNA-DNA hybridization (> 70%). Consequently, MS438^T is proposed as a new AAP species in the genus *Fulvimarina*.

This discovery of MS438 signifies a new insight into the *Fulvimarina* genus as well as AAPs. The presence of oxygen-dependent anoxygenic photosynthesis in a genus where the process has remained undetected is noteworthy and solidifies the prevalence of AAPs in *Aurantimonadaceae*. Additionally, such an exciting genomic integration of a potential *Rhizobium* phage could suggest a larger host range than usual, MS438 transducing genes from another bacterium and phage genes possibly playing a role in the evolution of some AAPs.

Section Type AEM

Abstract selected for Symposium 8 – Microbial Ecology & Sustainability June 18, 2026

Symposium 9 Abstracts/ Résumés du Symposium 9

396 - AMR and One Health in a Changing World

Simon Otto`[1, 2, 3]

[1]University of Alberta, School of Public Health, [2]Human-Environment-Animal Transdisciplinary (HEAT)-AMR Research Group, [3]Centre for Health Communities, School of Public Health, University of Alberta

We are at an important juncture in our battle to tackle the wicked problem of antimicrobial resistance (AMR). Surveillance is a vital component and is a strategic pillar in both the Pan-Canadian AMR Action Plan and the Alberta One Health AMR Framework for Action. We require representative data on AMR and antimicrobial use (AMU) from relevant One Health sectors to inform stewardship policies and programs. The environment is a focus of future surveillance efforts, while existing programs continue to have important gaps that must be addressed. Effective surveillance programs are based on clear objectives to ensure the data, information, and communication are fit for purpose. This talk will explore Canada's current integrated AMR/AMU surveillance systems relative to the exciting state of science. It will explore the importance of objectives to understand how we move forward with an effective One Health surveillance program that will meet our current and future needs. I will review our evaluation of Canadian AMR/U surveillance and our current research in food animal diagnostic testing as well as AMR surveillance in the agriculture and environmental sectors.

Section Type: AEM

Invited speaker in Symposium 9 – One Health, Climate Change, and Emerging Pathogens

9 - Across borders and hosts: One Health and a Superbug

Santiago Castillo-Ramirez[1]

[1]Universidad Nacional Autonoma de Mexico

Acinetobacter baumannii is a very important bacterial pathogen. Most of the genomic epidemiology of this bacterium has considered human clinical isolates. Recently, some studies have focused on *A. baumannii* from non-human sources. Yet, a comprehensive view of *A. baumannii* from different non-human sources is nonexistent. Amassing a huge data set (> 23,000 genomes), we generate an overarching view of non-human populations of *A. baumannii*. We noted that *A. baumannii* was recovered from 97 different sources and over 100 countries. Our diversity analyses unveil that non-human isolates are more diverse than the human ones and have an open pangenome. Even more, a population structure analysis and a phylogenomic tree reveal frequent transmission between humans and non-human sources, but also the existence of source-specific lineages. Our analysis of the resistome shows that a few sources (dogs, horses, and wastewater) not only have a similar number of antibiotic resistance genes (ARGs) compared to human isolates but also present highly clinically relevant beta-lactamases, such as NDM-1 or OXA-23. Finally, as a whole, non-human sources pose an open source of ARGs. Collectively, these analyses indicate that non-human *A. baumannii* is very diverse and could be a public health concern. On top of that, this work highlights the need for more research on *A. baumannii* under a One Health framework.

Section Type AEM

Invited speaker in Symposium 9 – One Health, Climate Change, and Emerging Pathogens

382 - Improving competitive exclusion in domesticated poultry

Zhixuan Feng[1], Jinha Suh[1], Yann Boucher[2], Frank Rheindt[2], Jennifer Ronholm[1]

[1]McGill University, [2]National University of Singapore

The gastrointestinal microbiota plays a central role in host health, productivity, and disease resistance in poultry. There has been interest in leveraging the microbiota as an alternative to antibiotics in industrial poultry production in recent years. If the gastrointestinal microbiota of domesticated chickens could be optimized to resist colonization by bacterial pathogens, perhaps the reliance on antibiotics for this purpose could be reduced. In this project, we hypothesized that a deep culturing approach, using several media and growth conditions, could be used to collect bacterial isolates representing a significant portion of the overall chicken gastrointestinal microbiome; and that at least some of these isolates could inhibit the growth of bacterial pathogens in a reductionist co-culture assay.

Fecal samples from six healthy Canadian laying hens as well as six wild Red Junglefowl from Singapore were collected and subjected to deep culturing and metagenomic sequencing. Fecal samples from Canadian hens were cultured using 76 different culture conditions and this generated 1240 bacterial isolates representing 128 bacterial species. Shotgun metagenomic sequencing of the same samples identified the presence of 545 bacterial species, revealing substantial discordance between cultured and sequenced taxa. The 12 optimal culture conditions for bacterial diversity, as identified from the data produced by Canadian laying hens, were used to deep culture wild Red Junglefowl fecal samples - which were shown to have significantly more bacterial diversity than domesticated chickens. Future, comparative bioinformatic analyses will quantify differences in microbial richness, taxonomic composition, and functional potential between wild and domesticated birds.

Deep culturing captured unique taxa which were not detected by metagenomics, including members of the *Comamonadaceae* and *Neisseriaceae*. Deep culturing followed by whole genome sequencing has also revealed extensive within-host strain diversity - which would not have been seen from metagenomics alone. This is particularly true among *Escherichia coli* populations, where several different sub-lineages appear to co-exist in almost all chickens. However, a large proportion of the microbiota—especially obligate anaerobes such as *Lachnospiraceae* and *Oscillospiraceae*—remain uncultured despite their apparent functional importance. These findings highlight both the limitations of current methodologies and the untapped potential of underexplored microbial reservoirs.

Several isolates obtained from wild and domesticated chickens have been tested in co-culture for the ability to inhibit the growth of *Salmonella enterica* ser. Typhimurium and avian pathogenic *Escherichia coli*. Some isolates have demonstrated a significant ability to inhibit the growth of these important bacterial pathogens.

The integration of these datasets is expected to yield a comprehensive culture collection of avian gut bacteria which are now available to the community, including several strains derived from wild hosts that may have been lost during domestication. This resource will support downstream functional studies, including mechanistic analyses of interbacterial interactions and probiotic formulation. More broadly, the work addresses a critical challenge in modern agriculture: reducing reliance on antibiotics while maintaining animal health and productivity. By identifying naturally occurring microbial antagonists of pathogens, this research contributes to the development of next-generation probiotics and advances a One Health approach to mitigating antimicrobial resistance.

Section Type AEM

Invited speaker in Symposium 9 – One Health, Climate Change, and Emerging Pathogens

210 - Microbial Community Dynamics Following Permafrost Thaw in the Canadian High Arctic: Linking In Situ and Laboratory Approaches

Chloe MacLean[1], Stephanie Coulombe[2], Ian Hogg[2], Bryan Vandenbrink[2], Jonathan Van Hamme[1], Eric Bottos[1]

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Microorganisms play a central role in regulating carbon release from permafrost ecosystems. Permafrost represents one of Earth's largest carbon reservoirs, as its frozen state protects stored carbon from microbial metabolism. However, as permafrost thaws in response to rising temperatures, previously sequestered carbon becomes bioavailable, fueling the production of greenhouse gases including carbon dioxide (CO_2) and methane (CH_4). The active layer, which lies above the permafrost and undergoes annual freeze-thaw cycles, harbours a microbial community that is structurally and functionally distinct from that of the permafrost due to differing environmental conditions. As the permafrost table deepens, the barrier between the permafrost and active layer microbial communities is removed, shifting selective pressures and promoting the mixing of microbial communities and nutrients between layers. This study examines the structural and functional responses of microbial communities to permafrost thaw under both laboratory and field conditions using soils collected from two field sites in the Canadian High Arctic, near Cambridge Bay, Nunavut: Augustus Hills and Long Point. Controlled laboratory microcosm incubation experiments were designed to examine the roles of environmental filtering and community mixing in shaping microbial responses. In parallel, a field-based transplant experiment was conducted to evaluate how these same soils respond to thaw in situ. Microbial community structure was assessed using 16S rRNA gene sequencing, while CO_2 and CH_4 flux were measured to investigate changes in greenhouse gas production. Pre-thaw microbial community composition differed significantly between the two field sites (PERMANOVA; permafrost: $p = 0.011$; active layer: $p = 0.004$). The results of the Augustus Hills microcosm incubations showed consistent significant shifts in microbial community composition following thaw in all treatments (PERMANOVA; $p < 0.05$). The results of the Long Point microcosms showed significant shifts in microbial community composition in the active layer-dominant microcosms only. These shifts in microbial community composition were accompanied by site- and treatment-specific differences in CO_2 and CH_4 production (ANOVA; $p < 0.05$). Analysis of the field-based transplant experiment samples is ongoing. This integrative approach will allow for the identification of key ecological drivers, including nutrient mixing and microbial dispersal, that govern microbial community responses to thaw. By directly comparing controlled laboratory and field-based experiments, this study evaluates the extent to which laboratory incubations replicate in-situ thaw dynamics. Linking post-thaw microbial community structure to greenhouse gas production will provide critical insight into the ecological processes driving changes in microbial communities and carbon release from thawing permafrost, improving predictions of permafrost ecosystem responses to climate change.

Section Type: AEM

Abstract selected for Symposium 9 – One Health; Climate Change and Emerging Pathogens

Symposium 10 Abstracts/ Résumés du Symposium 10

412 - Charting host-pathogen dynamics to boost immunity and combat antibiotic resistance

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[1]McMaster University

Antimicrobial resistance (AMR) is one of the most pressing challenges in modern medicine, yet many antibacterial discovery efforts continue to focus on bacterial growth under laboratory conditions that poorly reflect the host environment. During infection, bacterial pathogens encounter a complex landscape of immune pressures, nutrient limitation, antimicrobial peptides, oxidative stress, and inflammatory signals that profoundly influence both pathogen physiology and antimicrobial susceptibility. Understanding how pathogens sense, adapt to, and evade these host-derived cues offers new opportunities for therapeutic intervention. Our research program investigates the dynamic interplay between host innate immunity and bacterial adaptation using invasive non-typhoidal *Salmonella* (iNTS) as a model intracellular pathogen. We have developed complementary experimental and screening platforms to define how immune pressures reshape bacterial behavior and to identify new therapeutic strategies that exploit these vulnerabilities. These approaches include high-throughput chemical screening under host-mimicking conditions, macrophage-based infection models, and host-directed therapeutic discovery platforms designed to enhance cell-intrinsic antimicrobial defenses. A major focus of our work is understanding how innate immune mechanisms influence bacterial gene expression during infection. By combining macrophage infection models with bacterial transcriptomics in host cells deficient in specific innate immune pathways, we are beginning to map how distinct immune signals shape the *Salmonella* transcriptional landscape and drive adaptive responses associated with intracellular survival and antibiotic tolerance. These studies provide a systems-level view of host-pathogen communication and reveal infection-specific bacterial processes that are invisible under conventional laboratory growth conditions. This presentation will highlight recent advances from our laboratory demonstrating how infection-relevant screening environments uncover novel bacterial and host-directed therapeutic opportunities, how small-molecule combinations can overcome intrinsic antibiotic tolerance, and how charting host-pathogen interactions can reveal new strategies to enhance immunity and combat antimicrobial resistance. Together, these studies support a broader paradigm in which host-derived biology is leveraged as a roadmap for antimicrobial discovery.

Section Type I&I

Invited Speaker Symposium 10 – Microbiology in Health & Disease

411 - Decoding host metabolite control of C. difficile virulence guides therapeutic antitoxin design

Seam Miletic[1], Simoun Icho[1], John Tam[1], Peter Dosa[2], Casey Theriot[3], Roman Melnyk[1]

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The intestinal symptoms associated with *Clostridioides difficile* infection, including diarrhea, pseudomembranous colitis and life-threatening toxic megacolon, are mediated by the actions of the potent toxin TcdB on the colonic epithelium. Despite TcdB being directly implicated in disease symptom onset, asymptomatic carriage of TcdB-expressing strains is common, especially in hospitals and healthcare settings and in certain populations including newborns, suggesting that additional factors and conditions play a role in determining disease symptom severity. We hypothesized that host- and/or microbiota-factors may play a role in modulating disease severity by interacting with and inhibiting TcdB pathogenesis. To identify endogenous inhibitors of TcdB, we examined sterile fecal filtrates from healthy donors and found that the small-molecule fraction protected human cells from TcdB intoxication. Through a comprehensive follow-up we identified intestinal bile acids as the principal protective factors in the intestine. Biochemical studies revealed that bile acids directly bind TcdB and inhibit toxin-receptor interactions. Using cryo-electron microscopy, we demonstrated that bile acids bind within a hinge region of the toxin and stabilize a closed conformation that prevents receptor engagement. These findings establish bile acids as endogenous antitoxins that suppress TcdB activity through an allosteric mechanism. Together, these studies support a model in which disease outcome is determined by the balance between toxin burden and endogenous bile acid-mediated protection. Leveraging this natural defense mechanism, we developed gut-restricted bile acid analogues that protect against disease in preclinical models of *C. difficile* infection, providing a framework for the rational design of antitoxin therapeutics.

Section Type I&I

Invited Speaker Symposium 10 – Microbiology in Health & Disease

66 - The assembly of a hybrid type IV secretion system by a Crohn's disease-associated *Escherichia coli* strain

Jonas Wong[1, 2, 3, 4], Ahmad Refaat[1, 2, 3, 4], Pablo Villacampa-Teixeira[5, 6], Jonasz Patkowski[5, 6], Natalie Lapa[1, 2, 3, 4], Julian Ortiz[1, 2, 4], Kasia Dzierlega[1, 2], Blake Roberts[1, 2], Stephanie Tollenaar[7], Matthew Croxen[2, 8], Aducio Thiesen[9], Dina Kao[10], Xavier Clemente-Casares[1, 2], Ben Willing[7], Tiago Costa[5, 6], Wael Elhenawy[1, 2, 3, 4, 11]

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Type IV secretion systems (T4SSs) are central to bacterial pathogenesis. While traditionally known for facilitating DNA transfer via conjugation, T4SSs also mediate biofilm formation, which are critical for the fitness of adherent-invasive *Escherichia coli* (AIEC). AIEC are commonly isolated from Crohn's disease (CD) patients and are known to propel gut inflammation. Many AIEC strains carry F-like plasmids encoding the IncF subgroup of T4SSs. Unlike minimized systems that harbor 12 core components, the IncF family is expanded, having acquired additional genes that enhance conjugation. In this work (Wong et al., *Nat. Commun.,* 2025), we show that a biofilm-forming AIEC strain harbors an unusual IncF plasmid lacking two conserved components essential for T4SS functionality. This strain forms a natural hybrid T4SS where the missing plasmid components are supplied by a co-residing chromosomal T4SS present on an integrative and conjugative element (ICE). Using biochemical assays, we demonstrate that this functional machine is a mosaic of IncF and ICE-encoded proteins that cooperatively drive pilin polymerization and biofilm formation on epithelial cells. Furthermore, we show that a subpopulation of bacteria expresses the IncF and ICE-encoded genes in response to host cells, leading to the assembly of biofilms that promote AIEC fitness and exacerbate inflammation. These biofilms result in increased recruitment of immune cells and resist clearance compared to a T4SS-defective mutant strain. Together, these findings uncover crosstalk between two evolutionary distant mobile genetic elements to form a hybrid T4SS that mediates pathogenesis by a Crohn's disease-associated *E. coli* strain. This work is the first to comprehensively describe a novel hybrid T4SS, providing significant potential for developing new diagnostic and therapeutic strategies against CD-associated pathogens.

Section Type I&I

Abstract selected for Symposium 10 Microbiology in Health & Disease

274 - A High-Resolution Cryo-EM Structure of P66 Defines the Molecular Basis of Pore Formation and Adhesion in *Borrelia burgdorferi*

Nikolas Ewasechko[1], Yirui Guo[2], Elina Karimullina[1], Matthew Keough[1], George Chaconas[1, 3], Sven Bergstrom[4], Jenifer Coburn[5], Dominika Borek[2, 6], Alexei Savchenko[1]

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Borrelia burgdorferi is a spirochete that has evolved a complex enzootic lifestyle cycling between ticks and warm-blooded animal hosts. It is transmitted to humans through the bite of infected ticks and is the causative agent of Lyme disease—the most prevalent zoonotic, tick-borne infection in the Northern Hemisphere, with annual case estimates exceeding 300,000 in the United States alone. As a diderm (Gram-negative-like) bacterium, *B. burgdorferi* encodes dozens of outer membrane proteins that mediate nutrient acquisition, adhesion within vertebrate and invertebrate hosts, and evasion of host immune defenses; however, the specific functions and structures of most remain poorly characterized.

Here, we present the **2.2 Å cryo-EM structure of the integral outer membrane protein P66**—the first experimentally determined high-resolution structure of a member of this important protein group in *Borrelia*. Previous functional studies have shown P66 to be essential for murine, but not tick, infection and to interact with human β3 integrins, promoting endothelial activation. Moreover, P66 acts as a porin required for vancomycin uptake, although its physiological substrate remains unknown.

Our structural analysis reveals that P66 protomers adopt an atypical β-barrel architecture forming a stable trimer, strikingly distinct from the topology predicted by AlphaFold3. Mapping key functional residues onto this experimentally resolved **structure uncovers the molecular basis for P66's dual role as both an integrin ligand and a porin**.

Collectively, these findings provide the first molecular blueprint of this multifunctional outer membrane protein, offering new insight into *B. burgdorferi* pathogenesis and establishing a structural framework for future studies on substrate transport and structure-guided therapeutic design.

Section Type I&I

Abstract selected for Symposium 10 Microbiology in Health & Disease

37 - Developing an outer membrane vesicle-based vaccine against Klebsiella pneumoniae

Kaitlin Winter[1, 2], Ken H. Chu[1], Natalie Wilks[1, 2], Selina Cai[1, 2], Arissa Tejani[1, 2], Wei Ling Kuan[1, 2], Isabelle Hadad[1], Zeshawn Smikle[1, 2], Lianne Presley[1, 2], Tony Harn[1], Mandy Lo[1, 2], Manish Sadarangani[1, 2]

[1]Vaccine Evaluation Center, BC Children's Hospital Research Institute, Vancouver, Canada, [2]University of British Columbia, Vancouver, Canada

Background: *Klebsiella pneumoniae* is the highest priority bacterial pathogen according to the World Health Organization. In 2022, 60-70% *Klebsiella* isolates from hospitalized patients were resistant to at least 3 classes of antibiotics. Vaccination is a key strategy against antimicrobial resistance. Unfortunately, vaccine design against *K. pneumoniae* is complicated by high strain diversity. Conserved proteins across strains are potential vaccine candidates which may provide broad protection. Previously, we demonstrated that a homologous sublethal infection of dams with *K. pneumoniae* protects pups from a lethal challenge at 6 weeks old. We seek to develop an outer membrane vesicle (OMV)-based vaccine that protects against a variety of *K. pneumoniae* strains.

Methods: OMVs were purified from two significantly heterologous clinical isolates of *K. pneumoniae* - strains 61 (K24, O2a, sequence type [ST]1537), and 13 (KL114, O1ab,2a, ST219) - referred to as KP61 and KP13. DPBS or OMVs were delivered subcutaneously to 6-8-week-old female and male BALB/c mice, three times, three weeks apart (n=8, 1-2 repeats). As a positive control,10-12-week-old mice were infected with a sublethal dose of KP61 delivered intraperitoneally (n=8, 2 repeats). Sera were collected prior to each vaccination and infection and IgG/IgG1/IgG2a titers were evaluated by ELISA against KP61 and KP13 whole cell lysate. At 15-17-weeks-old mice were challenged with intraperitoneal KP61. Mice were monitored for up to 3 times daily and euthanized at humane endpoint. Antibody titers between groups were compared by either one-way ANOVA, with Dunnett's test, or two-way ANOVA, with Šidák's test to adjust for multiple comparisons, and survival after challenge between groups was compared using a Mantel-Cox log-rank test; $p<0.05$ was considered statistically significant.

Results: Vaccination with OMV61, OMV13, and sublethal infection with KP61 elicited KP61-specific IgG, IgG1 and IgG2a titers in both sexes ($p<0.001$ to DPBS). All three conditions also elicited KP13-specific IgG titers ($p<0.001$ to DPBS). OMV13 elicited higher IgG titers against 13 than 61 ($p=0.049$), while OMV61 and sublethal infection generated similar titers to the two strains. The IgG1/IgG2a ratio against KP61 in OMV13 vaccinated mice was skewed towards IgG2a compared to OMV61 vaccination and sublethal infection which elicited a skewed response towards IgG1 ($p=0.028$).

Upon challenge, sublethal infection with KP61 trended ($p>0.05$) towards protecting both sexes (F: 62.5% survival *vs.* 37.5% survival in DPBS controls; and M: 50% survival *vs.* 25%). Females vaccinated with OMV61 were similarly protected (62.5% survival *vs.* 37.5%), however males were not (33.3% survival *vs.* 25%). OMV13 provided no protection in either sex against heterologous challenge with KP61 (F: 12.5% survival *vs.* 37.5%; M: 37.5% survival *vs.* 25%).

Conclusions: Our work demonstrates that OMVs have potential as a protective vaccine against *Klebsiella pneumoniae*. Protection was limited in our model, requiring a higher sample size to achieve statistical significance. IgG titer did not appear to be a predictor of vaccine efficacy for *K. pneumoniae* infection. Further work is needed to elucidate the protective mechanisms elicited and how to exploit these to develop a broadly protective vaccine. A broadly effective *K. pneumoniae* vaccine would have high global impact against antimicrobial resistance.

Section Type I&I

Abstract selected for Symposium 10 Microbiology in Health & Disease

Symposium 11 Abstracts/ Résumés du Symposium 11

106 - Harnessing cropping system microbiomes

Matthew Bakker[1]

[1]University of Manitoba

The microbiomes that permeate cropping systems represent a vast collection of functional capacities, many of which have no analog in plants and no replacement among the typical management tools available to land managers. Included in this set are both beneficial functions (e.g., recycling of nutrients into plant-available forms, suppressing pathogens, etc) and undesirable functions (e.g., conversions of nitrogen fertilizer into gaseous byproducts, pathogenesis, etc). Thus, managing microbiomes offers a path to enhanced agroecosystem functionality and productivity. However, overwhelming complexity clouds the prospects for achieving the required degree of control. This presentation will describe examples of management-driven impacts on agricultural microbiomes, considering community structure as well as functional potential. Case studies will include microbiome responses to tillage and to enhanced fertilizers that include inhibitors of microbially-mediated transformations of nitrogen, as well as an isolate-based assessment of functional potential and application. Through this presentation, I hope you will come to see that microbiologists have important roles to play in advancing the productivity and sustainability of cropping systems.

Section Type: AEM

Invited Speaker for Symposium 11 - Agriculture & the Plant Holobiont June 18, 2026

103 - How Pangenomic Architecture Shapes Rhizobia Host Range and Specialization

Anna Simonsen[1]

[1]University of Alberta

Horizontally transmitted symbionts often form highly complex specialization patterns with their hosts. Bacteria are well known to have flexible genomes, with different strains carrying different gene content despite being very closely related or within the same species complex. However, it remains unclear how this flexibility in gene content impacts bacterial symbiont interactions with their hosts. In this presentation, I discuss how the architecture of bacterial pangenomes affects host range, specialization, and free-living stages in the legume-rhizobia mutualism, using the Australian hyperdiverse *Acacia-Bradyrhizobium* system as a model. *Bradyrhizobium* is a globally common rhizobia genus in soil that frequently switches hosts and recombines through horizontal gene transfer, making genetic signals of specialization more challenging to detect. Using this study system, my research has identified clear patterns of genome variation within a single nitrogen-fixing symbiont species (*Bradyrhizobium diazoefficiens*, n=375 strains) isolated from a single endemic Australian legume host (*Acacia acuminata*), which correlates strongly with abiotic conditions of the symbionts' soil origin - specifically, increasingly streamlined genomes with increasing abiotic stress. To test symbiotic function, these sequenced isolates were subsequently used to inoculate 17 *Acacia* species (from diverse ecoregions) in glasshouse experiments using a mixed culture of a strain subset (n=220), with strain occupancy quantified via root nodule metagenomics. Analysis of occupancy patterns revealed that larger symbiont genomes with higher numbers of protein-coding genes could infect a broader range of host species. Further analysis reveals genomic compartments of specialization uniquely associated with particular legume species, after controlling for strain phylogenetic background. Together, these results highlight the critical role of pangenomic variation in determining rhizobia host range, and suggest a tradeoff between adaptation to free-living and symbiotic life stages - reflected in gene content variation that drives either genome expansion or streamlining.

Section Type AEM

Invited Speaker Symposium 11 – Agriculture & the Plant Holobiont June 18, 2026

105 - Host and microbial control of arbuscular mycorrhiza symbiosis through peptide hormones

Lena Maria Mueller[1]

[1]Salk Institute

Arbuscular mycorrhiza (AM) symbiosis is a mutually beneficial interaction between plants and soilborne fungi of the *Glomeromycotina* subphylum. In this association, fungi provide mineral nutrients in exchange for plant-derived carbon, requiring tight control to maintain a balanced mutualism. Because AM fungi are obligate biotrophs and fully dependent on host-derived carbon, most research to date has focused on how plants regulate symbiosis. For example, the host controls the interaction through complex signaling networks that integrate symbiosis status and nutrient demand across colonized root cells and distal tissues. By contrast, the role of the fungal partner in regulating symbiosis has received much less attention. Our work shows that AM fungi actively interfere with endogenous host control pathways for their own benefit.

In summary, we found that several genes encoding secreted CLE (CLAVATA3/EMBRYO SURROUNDING REGION-related) peptides are upregulated in *Medicago truncatula* roots during AM symbiosis. The AM-induced MtCLE53 acts as a negative autoregulatory signal that restricts fungal colonization through the shoot-acting receptor-like kinase SUNN. We further found that the pseudokinase CORYNE, known to form a complex with SUNN, also functions as a negative regulator of AM symbiosis. However, CORYNE is only partially required for MtCLE53 signaling, suggesting that it integrates multiple signaling pathways. Consistent with this, our data indicate that CORYNE is part of an as-yet-unknown receptor complex that perceives a distinct symbiosis regulator, MtCLE16. In contrast to MtCLE53, MtCLE16 is induced in colonized cortex cells and promotes AM symbiosis by dampening host stress and defense responses. We hypothesize that MtCLE16 acts as a local signal amplifier that enhances symbiosis, in stark contrast to previously characterized plant CLE peptides, which predominantly function as negative regulators in this context.

Intriguingly, some AM fungi also produce signaling peptides resembling those of plants, including CLE-like peptides. Functional analysis of a CLE-like peptide from *Rhizophagus irregularis* shows that it signals through an as-yet-unknown host receptor complex involving CORYNE to manipulate host responses for microbial benefit. Together, our findings suggest that host and microbial control of AM symbiosis are highly intertwined, with AM fungi mimicking symbiosis-promoting CLE peptides to co-opt host signaling pathways for their own benefit.

Section Type MGCM

Invited Speaker Symposium 11 – Agriculture & the Plant Holobiont June 18, 2026

218 - An evolutionary and functional analysis of virulence gene promoters in *Pseudomonas syringae*

Racquel A. Singh[1], Tamar V. Av-Shalom[1], Bradley Laflamme[1], Marcus M. Dillon[1], Renan N. D. Almeida[1], Darrell Desveaux[1], David S. Guttman[1, 2]

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Microbial plant pathogens employ diverse strategies to infect and colonize their hosts, posing a major threat to global food security. These challenges are expected to intensify under changing environmental conditions that can alter host susceptibility and pathogen spread. While plants can mount robust immune responses upon pathogen detection, successful pathogens must evolve strategies to evade or suppress these defenses. Although extensive work has focused on how pathogens evolve their virulence-associated genes to escape recognition, far less is known about how they might **adjust the expression levels of these virulence genes** over evolutionary time in order to effectively evade host surveillance systems.

The bacterial phytopathogen *Pseudomonas syringae* infects hundreds of plant species worldwide and causes significant agricultural losses annually. The virulence of this pathogen is dependent on its type III secretion system, which it uses to inject virulence proteins, termed effectors, directly into plant cells. However, these same effectors can be recognized by intracellular immune receptors in resistant plant species, triggering effector-triggered immunity (ETI).

While effector diversification at the protein-coding level is well characterized, the role of effector regulatory variation in immune evasion remains poorly understood. We hypothesize that *P. syringae* modulates effector expression via sequence variation in effector promoters (“hrp box promoters”) to minimize immune activation. To test this, we identified and analyzed 9,126 promoters upstream of effector genes across 494 diverse *P. syringae* genomes. Phylogenetic and motif analyses revealed 574 unique sequences characterized by conserved core motifs and variable sites. To quantify functional variation, we constructed a library of over 220 promoter variants fused to a fluorescent reporter and optimized a high-throughput microplate assay to measure promoter activity. Our results demonstrate substantial variation in promoter strength, with specific polymorphisms significantly influencing effector expression levels. Ongoing work aims to assess how promoter variation impacts host-microbe interactions *in planta* and to identify evolutionary pressures shaping effector promoter diversity. Together, these findings will expand our understanding of how changes in pathogen regulatory sequences influence disease outcomes and may reveal potential molecular targets for improved disease management in crops.

Section Type MGCM

Abstract Selected for Symposium 11 – Agriculture & the Plant Holobiont June 18, 2026

Symposium 12 Abstracts/ Résumés du Symposium 12

110 - From Spectra to Solutions: Proteomics at the Forefront of Fungal Infection, Immunity, and Resilience

Jennifer Geddes-McAlister[1]

[1]University of Guelph

Fungal diseases impact the lives of millions of people across the globe ranging from superficial to systemic infections. Treatment options toward fungal diseases are limited given host cytotoxicity and availability, the emergence of new pathogens with intrinsic resistance and a heightened evolution toward resistant strains. To effectively combat fungal disease, my research team harnesses the cutting-edge power of mass spectrometry-based proteomics integrated with computational biology. By identifying protein drivers of fungal disease, we can provide new biological insights across four pillars of research: i) Prevention, ii) Diagnostics, iii) Monitoring, and iv) Treatment. For prevention, we disrupt critical proteins and pathways to weaken the pathogen and prevent infection; for diagnostics, we define dual-perspective protein production signatures, spanning both host and pathogen, across spatial and temporal dimensions to enable precise diagnostic and prognostic insights. For monitoring and treatment, we explore host-pathogen interactions at the protein level, uncovering novel druggable targets essential for therapeutic innovation, and we combat antifungal resistance through protein targeting to restore the efficacy of existing antifungal drugs. Together, our integrated proteomics-driven approach offers transformative solutions to fungal disease management with the goal of advancing global health initiatives.

Section Type: MGCM

Invited Speaker for Symposium 12 - Fungi as Nature's Engineers for Health, Environment, and the Sustainable Bioeconomy June 18, 2026

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414 - Towards a complete understanding of antifungal resistance

Christian Landry[1]

[1]Université Laval

Only a handful of antifungal molecules are regularly used in clinics and agriculture; resistance to these molecules can evolve during treatment or even segregate among naturally occurring populations of opportunistic pathogens. In a context where new drugs are slowly being developed, and drug targets are limited, it is imperative to develop approaches and strategies to understand resistance mechanisms, slow resistance evolution, and prevent the spread of mutations. We combine synthetic biology, high-throughput sequencing, and literature mining to fully document antifungal resistance mutations and quantify their fitness cost and cross-resistance. In this seminar, I will present our most recent work on this topic and how it can be used for precision antimicrobial treatment, and discuss how it could potentially be used to develop new drugs to overcome the most frequent resistance mutations

Section Type MGCM

Invited Speaker for Symposium 12 - Fungi as Nature's Engineers for Health, Environment, and the Sustainable Bioeconomy June 18, 2026

340 - Flushing Out the Mycobiome: Wastewater reveals distinct hospital and community fungal colonization patterns

Claire Kamaliddin[1, 2], Nicole Acosta[2], Jangwoo Lee[2], Barbara Waddell[2], Maria Bautista Chavarriaga[3], Chloe Papparis[3], Daniela Naomi De la Torre Espinoza[2], Emily Au[2], Kristine Du[2], Tanis Dingle[4], Julie Carson[5], Christine O'Grady[6], Rhonda G Clark[3], Bonita Lee[7], Judy Qiu[8], Xiaoli Pang[8], John M. Conly[2, 9], Casey RJ Hubert[3], Michael D Parkins[2, 9]

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Background: Wastewater-based surveillance (WBS) is a powerful, objective, and inclusive population-level approach for monitoring population health. Current programs predominantly focus on viral targets (SARS-CoV-2, influenza, RSV, etc.) or on antimicrobial resistance genes in bacteria; however, any microorganism or substance can be detected through WBS.

Candida spp are leading causes of bloodstream infections, disproportionally affecting immunocompromised hosts and associated with high morbidity and mortality. Surveillance of fungal infections, particular Candida, remains limited, and little is known about community versus health care-associated species distribution.

Methods: Composite 24-hour wastewater samples were collected from January 2022 to December 2023 from two tertiary care hospitals (700 and 650 inpatients), sampled twice per month, and one community wastewater treatment plant (servicing 1.08 million residents), sampled once per month in Calgary, Alberta. DNA was extracted from wastewater pellets using DNeasy PowerSoil kits (Qiagen). Paired-end sequencing (2 × 150 bp) was performed on the Illumina Novaseq 6000 PE150 platform, targeting ~10 gigabases (~33M paired reads/sample). Raw reads were quality filtered using FastQC (v.0.11.9) and trimmed with Trimmomatic (v0.39). Fungal taxonomic classification was performed using Kraken2 for species and genus identification, and Bracken for composition analysis, using FungiDB database for reference. Alpha and beta diversity analyses were conducted at the genus level using the vegan package (v2.7-3). To account for differences in fecal bioburden across samples, bacterial 16S rRNA gene abundance was used for normalization.

Results: Ninety-nine samples were sequenced and analyzed, including 22 from the community and 40 and 37 from each hospital site. Fungi were minor constituents of overall wastewater metagenomes, with a median of 124,953 (Q1-Q3 [109.929-168.381]) reads per sample, representing 0.15% of the total sequencing output. A total of 69 fungal genera and 127 species were identified consistently across all locations. The overall diversity and distribution varied between hospitals and communities. Alpha-diversity was lower in community wastewater relative to the two hospitals (Shannon index: 2.84±0.71 *vs.* 3.25±0.54 and 3.34±0.45, p=0.0003; Simpson index: 0.81±0.18 *vs.* 0.90±0.12 and 0.92±0.1, p=0.0002). The lower diversity in community samples was driven by an over-representation of the *Saccharomyces* genus commonly used in industrial processes such as brewing. Beta-diversity, assessed using Bray-Curtis analysis, showed a separation by collection site (p = 0.001).

Candida normalized abundance was higher within hospitals (0.535, $_{Q1\text{-}Q3}$ [0.286-0.830]) than in the overall community (0.170, $_{Q1\text{-}Q3}$ [0.139-0.197]), $p < 0.001$. In particular *Candida (p<0.001)*, *Nakaseomyces (including N. glabrata, formerly C. glabrata) (p<0.001)* and *Pichia (including Pichia kudriavzevii* (formerly *C. kruzei*)) ($p = 0.146$) genera were disproportionately abundant in hospital wastewater.

Conclusion: Wastewater mycobiomes differ markedly between hospital and community settings, with consistent enrichment of clinically relevant Candida species in acute care. These findings support near-to-source WBS as a scalable approach for real-time surveillance of fungal pathogens and a potential tool to track healthcare-associated infection risk at the population level.

Section Type: AEM

Abstract selected for Symposium 12 - Fungi as Nature's Engineers for Health, Environment, and the Sustainable Bioeconomy June 18, 2026

202 - Phages in fungal soup: the influence of fungi on phage-bacterial interactions

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Phages (bacterial viruses) are key drivers of bacterial community dynamics. However, the interaction between the phage and its bacterial host does not occur in isolation, but rather in complex communities consisting of other microbes, including eukaryotic ones such as fungi, which also compete for the same resources. As fungi are prolific producers of bioactive secondary metabolites, we sought to investigate whether fungal metabolites can directly influence phage infection to promote or interfere with growth of the bacteria. We screened phages infecting *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* in spent media generated from clinical strains of *Candida albicans,* an opportunistic human fungal pathogen. Most interactions we detected could be attributed to acidification of the medium and were not followed up on. However, fresh fungal medium (yeast peptone dextrose) inhibited infection of *E. coli* phage Mu, while spent medium of one *C. albicans* strain rescued this inhibition. Both the bacterial host and phage remained stable in the fresh and spent media, indicating the effect is specifically on phage infection. Substituting dextrose in the fungal medium with other carbon sources (fructose, galactose, glycerol, sucrose, and maltose) revealed that only fructose can fully recapitulate both the inhibition phenotype of fresh medium and rescue phenotype of the spent medium, hinting at a specific fungal response. Furthermore, adding dextrose back into the media revealed that phage inhibition and rescue are likely independent phenotypes, such that dextrose reduced phage titer in fresh medium, but the rescue phenotype was not observed when spiked into the spent medium. The phage infection rescue phenotype of fungal spent media was generalizable to other phages and bacterial hosts but remained phage-bacterium pairing specific. Protease treatment and heat denaturation of fungal spent media did not abolish the inhibition and rescue phenotype suggesting that the fungus is using a non-protein signal to influence phage infection. Overall, studying phage-bacterial dynamics in the context of other microbes, fungi specifically in this study, will improve our understanding of their interactions within complex natural environments.

Section Type AEM

Poster session & Abstract selected for Symposium 12 - Fungi as Nature's Engineers for Health, Environment, and the Sustainable Bioeconomy June 18, 2026

320 - Quantitative Genetic Analyses of Virulence and Antifungal Susceptibility Variations in the human fungal pathogen, Aspergillus fumigatus

Jezreel Dalmieda[1], Jianping Xu[1]

[1]McMaster University

Human fungal pathogens are lethal infectious agents, particularly in immunocompromised individuals. *Aspergillus fumigatus* is a leading cause of invasive aspergillosis, with mortality rates reaching up to 90%. Due to its prevalence, adaptability, and increasing antifungal resistance, the World Health Organization has classified *A. fumigatus* as a “Critical Priority” pathogen. Current treatment relies on triazole antifungals; however, resistance to triazoles is rising globally and threatens the effective management of invasive disease. Despite this, the broader fitness consequences and genetic mechanisms underlying triazole resistance remain poorly understood. In particular, it is unclear whether resistance is associated with trade-offs in pathogenicity-related traits such as virulence and thermotolerance. *A. fumigatus* has recently been identified as exhibiting the highest number of meiotic crossovers among all eukaryotes, resulting in highly complex genotype-phenotype relationships. Our objectives aim to **(1)** quantitatively analyze potential cost between triazole resistance and thermal growth, biofilm formation, and hemolysin production, **(2)** to characterize genome-wide SNP variation in *A. fumigatus* sexual crosses via recombination rates and segregation ratios, and **(3)** perform QTL mapping to identify specific loci associated to phenotypic traits. 528 F1 progeny were generated from five controlled crosses between parental strains divergent among various antifungal and virulent-related phenotypes. Phenotypic tests were performed among crosses to quantify antifungal susceptibilities, thermotolerance, and virulence-associated traits. Progeny were genotyped using a genotype-by-sequencing (GBS) approach, while parental strains were previously whole-genome sequenced (WGS) at high coverage. WGS SNPs were used to construct a high-resolution genetic linkage map for each cross. We identified multivariate structure and clustering overlap between triazole resistant and susceptible strains, consistent with extreme recombination and polygenic traits previously identified in *A. fumigatus*. We provide evidence of reduced virulent production among triazole resistant strains supporting a potential fitness cost of resistance. Finally, our crosses exhibit transgressive segregants with recombination and crossover distributions reflecting parental genetic backgrounds. These findings will contribute to a better understanding of the genetic architecture underlying triazole resistance and the role of recombination in shaping adaptive potential in *A. fumigatus*.

Section Type: MGCM

Abstract selected for Symposium 12 - Fungi as Nature's Engineers for Health, Environment, and the Sustainable Bioeconomy June 18, 2026

CSM MGCM Symposium/ Symposium SCM MGCM

A *Salmonella*-Specific Protein Enhances the Antimicrobial Peptide Response of the PhoPQ Two-Component System

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Salmonella enterica, a major global pathogen, has evolved a distinctive pathogenesis that differs from related enteric pathogens. A central regulator of Salmonella-specific virulence gene expression is the broadly conserved PhoP/PhoQ (PhoPQ) two-component system. PhoPQ serves a distinct role in Salmonella compared to other species, suggesting a need to adapt its function for its unique and expanded role in this species. In this talk, I describe a novel, *Salmonella*-specific component of the PhoPQ signalling system that our lab recently identified, which we have named PalA. I will discuss the discovery of PalA, as well as a suite of experiments we have performed to better understand how PalA impacts the PhoPQ system in Salmonella. Collectively, our data indicate that PalA serves to enhance PhoPQ activity when Salmonella encounter cationic antimicrobial peptides, thereby enhancing the expression of key Salmonella virulence factors under these conditions.

Section Type MGCM

Invited Speaker for MGCM Symposium June 19, 2026

231 - Tyrosine phosphorylation post-translationally regulates lipopolysaccharide biosynthesis in Enterohemorrhagic *E. coli*

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Enterohemorrhagic *Escherichia coli* (EHEC) O157 is a highly virulent zoonotic pathogen that is commonly associated with food, water, and livestock outbreaks. In humans, EHEC infection is characterized by acute gastroenteritis with bloody diarrhea that can lead to sever complications such as hemolytic uremic syndrome and kidney failure. A key virulence determinant of EHEC is the production of surface glycans, such as lipopolysaccharide (LPS) and group four capsule, which are essential for transiting and colonizing the gastrointestinal tract. LPS decorates the outside of the bacterial outer membrane and is a highly protective molecule that aids in thwarting host defences during infection, and is composed of three distinct parts: lipid A, core oligosaccharides, and O-antigen. The EHEC serotype O157 O-antigen is composed of the four-sugar unit *N*-acetylperosamine, fucose, glucose, and *N*-acetylgalactosamine, which is then repeated ~20-50 times within the complete LPS molecule. Recently it was shown that numerous O-antigen biosynthetic proteins were targets for tyrosine phosphorylation, however the role phosphorylation plays in LPS biosynthesis is completely unexplored. In our recent work, we show that the GDP-perosamine synthase PerA can be phosphorylated at positions Y7 and Y218, and mutation to the non-phosphorylatable or phosphomimetic mutants have divergent effects on LPS biosynthesis. EHEC Δ*perA* complemented *in trans* with PerA Y7F, Y7E, Y7F Y218F, and Y7E Y218E showed increased auto-aggregation, increased susceptibility to polymyxin B killing, and produced truncated LPS lacking the O157 antigen in comparison to EHEC Δ*perA* complemented with wild-type PerA. Furthermore, we tested the ability of PerA, and variants thereof, to catalyze the conversion of GDP-4,6-keto-deoxymannose into GDP-perosamine and observed impaired enzyme activity in the Y7F, Y7E, Y7F Y218F, and Y7E Y218E variants. Moreover, we used genetic code expansion to substitute Y7 and Y218 with the non-hydrolyzable phospho-mimetic of tyrosine, known as *p*-carboxymethylphenylalanine (*p*CMF), to better represent the phosphorylated state. The PerA Y7*p*CMF and Y218*p*CMF mutants also displayed impaired enzyme activity and produce little to no GDP-perosamine. To understand the role of phosphorylation in the active site we determine the structure of PerA in complex with pyridoxamine and alpha-ketoglutarate and pyridoxal phosphate and GDP to 1.90 and 2.10 Å, respectively. PerA Y7 is located in a central pocket that the coordinates the ribose and guanosine base of GDP, and Y218 is located near the active site within H-bonding distance to the beta-phosphate of GDP. Phosphorylation at either of these sites would likely impair the ability to properly bind to GDP-4,6-keto-deoxymannose, thus preventing catalysis. Our model supports a role for tyrosine phosphorylation in impairing LPS biosynthesis and extends our understanding of how bacteria might regulate surface glycan production in response to changing environmental or host colonization conditions.

Section Type MGCM

Abstract selected for MGCM Symposium June 19, 2026

26 - A Genetically Encoded Biosensor Facilitates Novel Insights into cyclic-AMP Dynamics and Heterogeneity in Mycobacterium tuberculosis

Srivathsa Shankar Kurpad[1, 2], Shaun Wachter[1], Amit Singh[2], Neeraj Dhar[1, 3, 4]

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3′,5′-Cyclic adenosine monophosphate (cAMP) is a nucleotide secondary messenger signaling molecule widely used across biological domains. *Mycobacterium tuberculosis* (Mtb), the etiological agent for tuberculosis, is unique in the prokaryotic world for its high dependence on cAMP signaling, with around 1% of its genome predicted to interact with cAMP. cAMP signaling in *Mycobacterium tuberculosis* is known to regulate the utilization of host-relevant carbon sources, virulence, resuscitation from dormancy, stress adaptation, and antibiotic tolerance. Additionally, Mtb seems to impose a dynamic, yet tight, regulation over its cAMP levels across the different stages of its infection cycle. While it has been implicated in several crucial physiological processes, its temporal dynamics and heterogeneity remain unexplored. A major limitation to studying cAMP signaling in mycobacteria is the lack of a reliable and reproducible method to monitor its levels in real time. The most prevalent methodologies employed to quantify cAMP levels in Mtb, or host cells, include ELISA, luminosity, radioimmunoassay-based detection mechanisms, or mass spectrometry. These detection methods, while accurate, require long sample preparation times, are incompatible with microscopy or cell sorting applications, and do not confer the ability to assay cAMP levels in real time or at the single-cell level. To address these issues and to better understand aspects of cAMP signaling in Mtb, we re-engineered a eukaryotic cAMP-specific fluorescent biosensor to optimize its use in the mycobacterial system. The use of this biosensor, named rmgCarvi, is the first of its kind in any prokaryotic system and it affords us the ability to investigate cAMP dynamics both at bulk and the single-cell level, and in real-time when leveraged in combination with flow cytometry and time-lapse fluorescence microscopy. Using rmgCarvi, we report a strong positive correlation between cAMP levels and growth rate in axenic cultures of *Mycobacterium tuberculosis*. We demonstrate a rapid and large induction of intramycobacterial cAMP upon resuscitation from dormancy that precedes growth resumption. Single-cell analysis revealed a stable bimodal distribution of intramycobacterial cAMP levels during dormancy separated by a zone of exclusion, and strong correlations between the extent of cAMP induction and time to re-growth to the intramycobacterial cAMP levels during dormancy. Upon infection in macrophages, we demonstrate a strong cAMP induction which is followed by a reduction in intramycobacterial cAMP levels in murine, but not human, macrophages, likely indicative of a cAMP burst into the macrophage in a cell-type dependent manner. Through our results, we demonstrate rmgCarvi's powerful utility in facilitating new experimental avenues to better understand the dynamics and heterogeneity of cAMP signaling in mycobacteria under diverse conditions, and in enabling the identification of novel players in the cAMP signaling network.

Section Type

Abstract selected for MGCM Symposium June 19, 2026

36 - Genetic Interaction Landscape of the Bacterial Essentialome

Zoe Istace[1], Arinnia Manjaly[1], Ali Hosseinnia[1], Sun Young Kim[1], Sadhna Phanse[1], Shubbham Dutta[1], Kevin Dang[1], Omara Firdous[1], Sakib Hossain[1], Hiroyuki Aoki[1], Tatiana Saccon[1], Alla Gagarinova[2], Khaled Aly[1], Mohan Babu[1]

[1]University of Regina, [2]University of New Brunswick

Essential genes required for cell survival and viability are highly conserved across bacterial species. Model organisms such as *Escherichia coli* provide a valuable system for studying gene-gene (genetic or epistasis) interactions, functional dependencies, and pathway cross-talk. Our high-throughput epistatic screening platform, the *E. coli* synthetic genetic array (eSGA), enables the simultaneous measurement of thousands of genetic interactions. Previous strategies to perturb essential genes have included CRISPR interference and a degron system based on ssrA tagging. In this study, as in yeast, we employed the Decreased Abundance by mRNA Perturbation (DAmP) strategy to generate *E.coli* strains with reduced expression of essential genes (hypomorphics). These alleles were validated for effects on gene dosage, growth, translation, morphology, and drug-target discovery. We then used the eSGA platform to generate digenic hypomorphic combinations by mating each F^- recipient essential DAmP strain with a high-frequency recombination donor essential DAmP allele. The resulting double hypomorphic mutants were imaged, and their colony growth fitness was quantified, revealing functional dependencies among essential genes and pathways. Using these genetic interaction data, we developed a mechanistic model linking a previously uncharacterized gene to cell divisome assembly. Together, our results provide a genome-wide framework of essential genetic interactions in bacteria, offering a community resource for dissecting essentialome biology and identifying conserved vulnerabilities with potential to inform antibacterial discovery.

Section Type

Abstract selected for MGCM Symposium June 19, 2026

171 - Streptomyces toxins: contributions to bacterial 'exploration' and antimicrobial activity

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Streptomyces are soil bacteria with a complex developmental cycle and a remarkable metabolic capacity: they are a major source of clinically important antibiotics. In addition to producing antibiotics as a defense mechanism, there is increasing evidence that these organisms are also capable of producing diverse toxins. We have data suggesting that during 'exploratory growth' by *Streptomyces venezuelae* - where 'exploration' is a recently discovered growth mode for these organisms - many large toxin-encoding genes are highly expressed. Deletion of the regulatory gene *quiK* resulted in both enhanced exploration and downregulation of many of these toxin genes, suggesting that *quiK* may be responsible for controlling a network of toxin-associated genes, in addition to influencing exploratory growth. We are working to determine whether these toxins impact *Streptomyces* exploration, how they exert their toxic effects, and how they contribute to *S. venezuelae* fitness when competing with microbes and other organisms in the soil. AlphaFold and sequence analyses indicate that many of these toxins appear to be substrates of the type VII secretion system (T7SS). As a first step, we have deleted the T7SS gene cluster in *S. venezuelae* and found that the T7SS mutant strain showed enhanced exploration on yeast extract-peptone (YP) media. Additionally, the T7SS mutant exhibits impaired growth when grown adjacent to the yeast *Saccharomyces cerevisiae* on solid media, indicating reduced competitive fitness in the absence of this secretion system (and presumed inability to deliver these toxins). To identify whether the T7SS substrates impact other microbial species, we conducted competition assays against wild-type *Streptomyces* and other soil microbes, such as *Bacillus subtilis.* The T7SS gene cluster encodes the effectors EsxA and EsxB, as well as the developmental regulator BldB. To differentiate the effects of losing (toxin) secretion capabilities, versus those stemming from loss of *esxAB* and *bldB*, we have reintroduced these three genes into the T7SS-deficient background. We are currently evaluating this strain's competitive fitness and exploratory behavior. Future work will involve using mass spectrometry to identify T7SS-specific substrates within the culture substrate by comparing the secreted proteome of the wild-type strain and the T7SS mutant. Additionally, we aim to characterize the mechanisms of action for these exploration-associated toxins, beginning with an assessment of whether they exert their effects on other microbes intracellularly or extracellularly. In all, these studies are shedding light on both an understudied secretion system and a unique group of toxic weapons available to *Streptomyces* for competition with other organisms.

Section Type

Abstract selected for MGCM Symposium June 19, 2026

372 - SLAM: Because Surface Lipoproteins Can't Just Walk Through Membranes

Tiana Lee[1], Dixon Ng[1], Megha Shah[1], Christine Lai[1], Trevor Moraes[1]

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Gram-negative bacteria are adaptable organisms that thrive in diverse environments. To do so, they have evolved surface lipoproteins (**SLPs**) to acquire nutrients, evade host immune defences, facilitate adhesion to target cells, and break down macromolecules through enzymatic activities. These proteins are produced in the cytoplasm and must reach the bacterial cell surface to function; consequently, bacteria have evolved many different secretion systems to shuttle these proteins across the periplasmic space and bacterial outer membrane. The type XI secretion system (**T11SS**) is a two-partner system comprised of an integral outer membrane beta-barrel translocon Slam (**S**urface **L**ipoprotein **A**ssembly **M**odulator) and one or several corresponding SLP substrates. Even though many gram-negative bacteria express multiple Slam homologs, the translocated substrates are highly specific to their corresponding Slam. Furthermore, deletions of either Slam or its substrate significantly reduce virulence in the bacterial pathogen *Neisseria meningitidis*, demonstrating the importance of the T11SS as a key virulence factor. While we have a general understanding of how Slam and its substrates function in bacteria, the precise molecular mechanism for substrate recognition and substrate translocation is not fully elucidated. **We are using a protein engineering and structure-guided approach to identify how each functional domain of Slam coordinates specificity and translocation of its substrate.**

Slam has two functional domains, an N-terminal periplasmic domain and a C-terminal beta-barrel domain within the outer membrane. To pinpoint the specific domain of Slam dictating specificity, we generated a library of hybrid Slam proteins by swapping the periplasmic domains between the three Slam homologs found in *Neisseria gonorrhoeae*. We adapted these neisserial Slam homologs and their corresponding substrates in *Escherichia coli* and then proceeded to test these hybrid Slam proteins in the same system. Our initial findings demonstrate that the N-terminal domain (NTD) is critical for translocation and confers specificity, the exception being the Slam3 NTD hybrid translocons, which facilitated translocation of all Slam substrates in varying degrees.

While the Slam translocon can recognize substrates with high specificity, we have yet to decipher the molecular mechanism behind the substrate translocation process. To identify how these two domains facilitate translocation at the molecular level, we are using cryo-electron microscopy (cryo-EM) to obtain high-resolution structures of Slam in different states of the translocation process. Using purified Slam embedded in detergent micelle, we obtained preliminary 2D classes of Slam particles, which allow us to validate the overall architecture and orientation of the two domains of the translocon. These structural states will provide insight into a novel translocation mechanism of key virulence factors in many gram-negative pathogens. This will provide a basis for the development of novel therapeutics used to inhibit T11SS-mediated virulence and pathogenicity.

Section Type MGCM

Poster session & Abstract selected for MGCM Symposium June 19, 2026

CSM I&I Symposium/ Symposium SCM I&I

142 - David vs. Goliath: How Minimalist Phage Satellites Take Over the Helper Viral Capsid

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Antimicrobial resistance is driving renewed interest in phage-based therapies. During infection, bacteriophages replicate and package their DNA into capsids via a terminase-mediated process. This step can be hijacked by phage satellites, mobile genetic elements that exploit phage particles for their own transmission. However, the mechanisms governing DNA packaging competition between phages and satellites remain poorly understood, particularly in newly discovered phage satellites.

We previously characterized a new class of phage satellites in *Vibrionaceae,* termed Phage-Inducible Chromosomal Minimalist Islands (PICMIs). Our model PICMI encodes only six genes and lacks canonical features associated with efficient parasitism, such as capsid remodeling or dedicated packaging machinery. With its original helper phage, PICMI hijacks only ~10% of capsids, and its mechanism of DNA encapsidation remained unresolved.

To identify determinants of packaging efficiency, we screened a large collection of natural *Vibrio* phages for susceptibility to PICMI parasitism. We uncovered a broad spectrum of outcomes, with some phages showing up to 80% of capsids containing PICMI DNA. This variation provides a powerful framework to dissect the molecular basis of satellite packaging.

Focusing on a highly susceptible phage, we identified Up1, the only PICMI protein with no assigned function, as a key determinant of packaging. Introduction of a stop codon in this gene reduces PICMI encapsidation, whereas its expression redirects packaging toward PICMI DNA, impairing phage production and infectivity. Structural predictions suggest that Up1 interacts with the phage terminase, supporting a model in which PICMI biases DNA packaging specificity, now being validated through biochemical and two-hybrid assays.

Together, our results show that a single protein can drive efficient capsid hijacking in a minimalist satellite. More broadly, PICMI-phage interactions form a continuum of parasitism efficiency shaped by ongoing co-evolution, with phage escape from parasitism as a key determinant.

Section Type

Abstract selected for I&I Symposium June 19, 2026

148 - Determination of Leptospira Lipid A Diversity Across Distinct Environments

Sophie Culos[1], Kenneth Weke[2], Zoey Ngu[1], Abanoub Mikhael[2], David R. Goodlett[1, 2], Helena Petrosova[1, 2]

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Pathogenic *Leptospira* are the causative agents of leptospirosis, an emerging zoonosis affecting humans, livestock, and wildlife across the globe. During infection, the host immune system engages with *Leptospira* lipopolysaccharide (LPS) and its constituents (O-antigen, oligosaccharide core, and lipid A). The structure and composition of LPS are central to pathogenicity; in other bacteria, modifications to LPS enable immune evasion, antimicrobial resistance, and adaptation to the host environment.

Leptospira are fastidious bacteria that require complex media for successful culture *in vitro*. They grow most readily in traditional EMJH medium, which falls short of reproducing a “host-like” environment. Alternatively, pathogenic *Leptospira* can be cultured in supplemented EMEM tissue culture medium. We have revealed that lipid A of the model pathogen *L. interrogans* grown in EMEM, and not in the traditional EMJH, more closely resembles lipid A found in infected host tissues. These results suggest that EMEM could be a translationally valuable model for studying LPS-related pathogenesis and *Leptospira* adaptation to the host environment.

In this study, both media were used to characterize lipid A modifications in *Leptospira*. Bacteria were cultured in EMJH (30°C and 37°C, 5% CO_2) or EMEM (37°C, 5% CO_2). Four representative pathogens (**P1+**), four species of unknown pathogenic potential (**P1-**), and two “intermediates” that may cause mild disease (**P2**) were studied. Lipid A from cell pellets was extracted using the Fast Lipid Analysis Technique (FLAT), a workflow that allows detailed lipid A characterization without lengthy solvent-based extraction and purification. Liquid chromatography tandem mass spectrometry-based label-free quantitative proteomic analysis was used to investigate leptospiral protein expression. In-house R-based workflows were developed for data analysis.

All screened pathogenic species followed the pattern of lipid A modification previously observed in *L. interrogans*. In EMEM medium, longer fatty acyl chains were incorporated into lipid A, and a portion of lipid A molecules lacked one acyl chain (penta-acylated lipid A). In contrast, lipid A from both P1- and P2 species in EMEM incorporated shorter fatty acyl chains and remained hexa-acylated. These structural differences likely result from differences in the specificity and expression of enzymes involved in LPS biosynthesis between pathogenic and non-pathogenic *Leptospira*.

Proteomics was employed to examine changes to expression of known and putative LPS biosynthesis enzymes. Preliminary analysis revealed that *L. interrogans* cultured in EMEM differentially expressed ~500 proteins, including those involved in LPS biosynthesis and virulence. Proteomics data from other representative *Leptospira* species are now being examined. The goal is to discern how culture in EMEM media affects protein expression across species with distinct pathogenic potential. Pathogenic-specific mechanisms will be distinguished from enzymatic pathways required for EMEM adaptation and screened for novel homologues involved in LPS biosynthesis.

This work will clarify how distinct lipid A structures offer an adaptive advantage across culturing environments. It is the first comprehensive study on functional LPS modifications in *Leptospira*, uncovering how these changes may impart advantage for pathogenic survival in the host.

Section Type

I&I

190 - Conformational dynamics and allosteric regulation of a tripartite efflux pump support substrate transport and antibiotic resistance

Evan Mercier[1], Mikayla Khammar[1], Rica Kabayiza[1], Chloé Girouard[1], Alexandre Gagné[1], Olivier Perreault[1], Louis-Patrick Haraoui[1], Louis-Charles Fortier[1]

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Tripartite multidrug efflux pumps span the inner and outer membranes of Gram-negative bacteria, export antibiotics and other cargo, and are key contributors to antibiotic resistance. Bacteria possess multiple different tripartite efflux pumps with broad and overlapping antibiotic specificities. This poses a significant challenge to understanding the role of individual transporters in antibiotic resistance. To address this challenge, we combine functional studies and gene-expression analysis in bacteria with mechanistic and structural characterization of individual efflux pumps in a purified *in vitro* system. This integrative approach enables us to unify physiological roles in bacteria with mechanistic and molecular-level details.

We have identified a limited set of tripartite efflux pumps in clinical isolates of the critical priority pathogen *Acinetobacter baumannii*, with expression patterns that indicate a role in resistance to the reserve antibiotic tigecycline. Their contribution to resistance was validated in adaptive laboratory evolution experiments where overexpression of *adeAB* was found to be the main driver of tigecycline resistance and was accelerated by expression of several other tripartite efflux pumps.

We have reconstituted one of these pumps, the ATP-powered transporter MacAB-TolC, *in vitro* from purified protein subunits. Using ATPase and real-time *in vitro* transport assays, we have validated its antibiotic cargo, including tigecycline, thus supporting its role in tigecycline resistance. In addition, we find that phospholipids are key activators of efflux and that the MacA subunit contributes to substrate selection and promotes efficient use of ATP. Distance-dependent fluorescence measurements (FRET) reveal that phospholipids and MacA act as allosteric regulators of MacB by supporting nucleotide-dependent conformational changes that drive antibiotic efflux. These structural and mechanistic details provide key insights into how efflux pumps are regulated, how antibiotic cargo is selected, and how these pumps can be inhibited.

Section Type

Abstract selected for I&I Symposium June 19, 2026

204 - Adeno-associated virus delivery of anti-alpha toxin monoclonal antibodies confers protection against *Staphylococcus aureus* infections

Josefien Hommes[1], Madison Hughes[2], Derek Cheung[1], Björn Petri[1], Lindsey Orthner[1], Tristan van der Linden[3], Bart Bardoel[3], Simran Deo[1], Deepak Patel[1], Ronald Flannagan[4], David Heinrichs[4], Sarah Wootton[2], Bas Surewaard[1]

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Staphylococcus aureus is a major cause of infections, ranging from mild skin conditions to severe life-threatening systemic diseases. Despite decades of effort, vaccine development has been unsuccessful, highlighting the need for alternative approaches. One promising candidate is MEDI4893 (Suvratoxumab), a monoclonal antibody that neutralizes α-toxin (AT), a key virulence factor with diverse functions including cellular lysis and induction of platelet aggregation during sepsis. MEDI4893 has demonstrated efficacy in various preclinical models of infection and showed encouraging results in a phase IIb clinical trial for ventilator-associated pneumonia. However, as an exogenous antibody, MEDI4893 is limited by its short half-life, high production costs, and restricted availability. To overcome these challenges, we investigated vectored immunoprophylaxis as a novel strategy for long-term protection. This approach uses an adeno-associated viral vector (AAV) to deliver the MEDI4893 gene, enabling sustained *in vivo* antibody expression. Following AAV-MEDI4893 administration, mice developed high and stable antibody levels in plasma and detectable titers at mucosal surfaces. Using intravital microscopy, we observed that vector-mediated MEDI4893 effectively prevented AT-induced platelet aggregation and microvascular thrombosis, thereby protecting animals from the lethal effects of intravenous toxin challenge. Infection studies confirmed that this protective effect extended to clinically relevant models. In murine pneumonia models, treated mice showed improved survival and reduced sickness behavior despite similar bacterial burdens, while in skin infection models, they were protected from dermal necrosis and exhibited lower bacterial loads. These findings highlight that AT is a major driver of pathology across multiple tissues and that its neutralization can mitigate disease severity. Together, our results demonstrate that AAV-mediated delivery of MEDI4893 provides durable, protective antibody levels and effectively neutralizes AT *in vivo*. This strategy represents a cost-effective, long-lasting alternative to traditional monoclonal antibody therapy and offers a promising prophylactic approach to mitigate *S. aureus* infections in the absence of a vaccine.

Section Type

Abstract selected for I&I Symposium June 19, 2026

282 - Secreted metalloprotease activity from vaginal *Prevotella bivia* dysregulates endothelin signaling in infection-induced preterm labour

Julia Nelson[1], Ana D'Aubeterre[2], Laura Sycuro[2, 3, 4, 5], Karen Lithgow[1]

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Bacterial vaginosis (BV) is a prevalent dysbiotic vaginal condition that increases the risk for health complications. In pregnancy, select BV-associated *Prevotella* species can vertically migrate to the uterus and have been implicated in triggering fetal membrane rupture and preterm labour. However, we lack mechanistic understanding of how *Prevotella* species contribute to adverse pregnancy outcomes. Vaginal *Prevotella bivia* encodes an M13 metalloprotease called PepO that exhibits sequence and domain similarity to a human metalloprotease called endothelin converting enzyme 1 (ECE-1). Human ECE-1 is a labour-activating protease that contributes to labour onset by cleaving the precursor peptide big endothelin-1 (bET1) into bioactive endothelin-1 (ET1), which induces uterine contractions via endothelin receptor signalling. We hypothesize that *P. bivia* PepO proteolyzes bET1 into bioactive ET1, thereby mimicking the activity of human ECE1 to initiate uterine contractile signalling and premature labour.

Comparative sequence analysis of *P. bivia* PepO and human ECE-1 revealed 43% sequence identity within the catalytic C-terminal M13 protease domain, 100% conservation within the substrate-binding domain (VNAYY), and 80% conservation within the metalloprotease active site motif (HExTH). Recombinant *P. bivia* PepO was expressed using a cell-free *in vitro* transcription-translation system and affinity purified with anti-FLAG magnetic beads. Proteolysis was quantified from purified recombinant *P. bivia* PepO using a fluorophore-quenched substrate specifically designed to measure ECE-1 activity. Purified *P. bivia* PepO proteolyzed the ECE-1 substrate with a reaction rate that was 4-fold faster than the reaction rate for recombinant human ECE-1. Inclusion of phosphoramidon, a known ECE-1 inhibitor, reduced PepO protease activity by 82% and site-direct mutagenesis of the *P. bivia* PepO active site (HExxH to HAxxH) abolished protease activity against the ECE-1 substrate. Next, cell-free supernatants from *P. bivia* were tested against the ECE-1 substrate. Supernatants from *P. bivia* were also found to proteolyze the ECE-1 substrate and phosphoramidon reduced this activity by 36%. To assess the proteolytic conversion of bET-1 to ET-1, cell suspensions of *P. bivia* were co-incubated with the bET-1 precursor over a time-course. SDS-PAGE analysis confirmed bET1 degradation by *P. bivia* after two hours, which also corresponded to detection of the bioactive breakdown product, ET-1, using an immunoassay that specifically detects ET-1, but not bET-1. Altogether, our findings confirm that *P. bivia* PepO exhibits functional mimicry of the human labour-activating protease ECE-1. This work identifies a new mechanism through which *P. bivia* could induce or augment labour by inappropriately activating contractility signaling pathways through bET-1 proteolysis.

Section Type

Abstract selected for I&I Symposium June 19, 2026

370 - Host innate immune responses promote T4SS-dependent biofilm formation by Crohn's disease-associated *Escherichia coli*

Natalie Lapa[1, 2, 3, 4], Ahmad Refaat[1, 2, 3, 4], Jonas Wong[1, 2, 3, 4], Stephanie Tollenaar[5], Ben Willing[5], Xavier Clemente-Casares[1, 2], Wael Elhenawy[1, 2, 3, 4, 6]

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Crohn's disease (CD) is a chronic inflammatory bowel disease of unknown etiology, characterized by relapsing bouts of inflammation and dysbiosis of the gut microbiome. CD-associated dysbiosis is marked by a decrease in microbial diversity and the expansion of pathobionts, such as adherent-invasive *Escherichia coli* (AIEC) strains. AIEC strains are isolated more frequently from CD patients compared to healthy controls. These bacteria have been shown to induce colitis in preclinical murine models of infection; thus, they fulfill Koch's postulates as a potential cause of CD-associated inflammation. AIEC strains are well-adapted to the gut environment; they adhere to and invade the gut epithelium, survive phagocytosis by macrophages, and outcompete commensals. A transposon mutagenesis screen previously identified a hybrid type IV secretion system (T4SS) in the AIEC strain NRG857c as essential for its persistence in the gut. Encoded by the *tra* operon, the T4SS spans the cell wall of AIEC and elaborates pili, which are known to mediate effector protein secretion and conjugation. In addition, these pili enable the bacteria to self-aggregate and form biofilms on the surface of epithelial cells. These biofilms can shield AIEC from eradication by the host immune cells. Notably, although the T4SS is minimally expressed by AIEC under standard laboratory conditions, it is significantly upregulated in the murine gut, suggesting that host-derived factors trigger its expression.

Given that AIEC thrives under inflammatory conditions, and that the T4SS is essential for AIEC persistence in the gut, we investigated whether the inflamed gut environment upregulates T4SS expression in AIEC. To this end, we pre-treated specific pathogen-free (SPF) mice with antibiotics to induce microbiome stress and subsequent inflammation. T4SS expression was induced in antibiotic-treated SPF mice, unlike untreated controls or germ-free animals. To further study the role of inflammation in upregulating T4SS, we employed an *in vitro* infection model. Preliminary data using this model indicated that the *tra* operon is upregulated in AIEC in the presence of intestinal epithelial cells (IECs), and that AIEC forms biofilms on the surface of IECs in a T4SS-dependent manner. Pivoting on our results *in vivo*, we sought to determine whether host innate immune responses play a role in triggering T4SS-dependent biofilm formation on IECs. In this regard, we inhibited the activity of the pro-inflammatory transcriptional activator nuclear factor kappa B (NF-kB) in IECs infected with AIEC. Using immunofluorescence, we found that AIEC forms fewer biofilms on the surface of IECs when NF-kB is inhibited. Together, these data suggest that host-derived cues can trigger the expression of the T4SS and biofilm formation in AIEC, thus giving insight into the pathophysiology of a Crohn's-associated pathogen.

Section Type

Abstract selected for I&I Symposium June 19, 2026

CSM AEM Symposium/ Symposium SCM AEM

11 - Listening to the Microbes: Comparative Responses to Amendment Strategies in Groundwater Bioremediation

Sampurna Nandy[1], Sydney Kennedy-Flynn[2], Petr Kuznetsov[1], Miles Dyck[2], Ania Ulrich[1]

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Petroleum hydrocarbons (PHCs) and organohalides (OHCs) are persistent pollutants that frequently coexist in groundwater systems. Their co-contamination can limit natural attenuation by generating complex redox conditions and metabolic stress. Biostimulation (nutrient addition) and bioaugmentation (the addition of specialized microbial cultures) are usually employed for accelerating remediation. While conventional geochemical monitoring is required for performance evaluation, bioassays can be used to assess microbial responses which ultimately drive remediation through microbial biomass and enzymatic action. However, our understanding of how microbial systems respond functionally to these different amendment strategies remains limited. Linking microbial biomass, enzymatic activity, and metabolic capacity is essential for evaluating remediation performance beyond conventional geochemical monitoring. This study evaluated microbial responses under different amendment strategies at two contaminated groundwater sites in Alberta, Canada. **Site A**, impacted by mixed PHCs and OHCs, received sequential nutrient biostimulation using a C:N:P amendment blend followed by bioaugmentation with a microbial consortium (Biologix; dominated by *Pseudomonas* spp.). **Site B**, a commercial groundwater site primarily impacted by PHCs, received bioaugmentation alone. Groundwater samples were collected from injection and monitoring wells at baseline and post-treatment intervals to assess microbial dynamics. Contaminant concentrations were monitored using gas chromatography, and field parameters, including pH, electrical conductivity, groundwater level, and well depth, were recorded. Microbial responses were evaluated using enzymatic activity assays for dehydrogenase activity (DHA) and catechol dioxygenase activity, qPCR targeting total bacterial 16S rRNA genes and *Dehalococcoides*, and community-level physiological profiling (CLPP). Functional shifts in microbial metabolism were further evaluated using non-metric multidimensional scaling (NMDS). It was found that the groundwater systems at both sites showed measurable microbial activity after amendment addition; however, their responses differed. At **Site A**, where the combined bio-strategy was applied, DHA and catechol dioxygenase activities increased more than baseline levels, indicating enhanced microbial respiration and a greater potential for aromatic hydrocarbon degradation. qPCR analysis further demonstrated substantial increases in total bacterial abundance following treatment, consistent with nutrient-supported biomass growth. In contrast, **Site B,** which received bioaugmentation alone, showed moderate increases in enzymatic activity and microbial abundance. Although microbial activation was evident, responses were more spatially variable across monitoring wells at **Site A** than at **Site B** during peak microbial activity. CLPP analysis of the sites showed broader substrate utilization at Site A than at Site B. Furthermore, estimation of NMDS analysis revealed a stronger separation between baseline and post-treatment samples at **Site A** compared to **Site B,** suggesting a stronger effect when nutrient supplementation accompanied bioaugmentation. In addition, a higher qPCR abundance targeting *Dehalococcoides* at **Site A** indicated enrichment trends consistent with reductive dechlorination potential, supporting the presence of multiple biodegradation pathways. Overall, the nutrient-supported treatment promoted stronger and more coordinated microbial activity than bioaugmentation alone. Multiple microbial indicator tests collectively confirmed stronger microbial activation when nutrients were added with microbes. These findings demonstrate that integrating enzymatic assays, molecular quantification, and functional profiling enhances the interpretation of remediation performance and complements conventional geochemical monitoring in complex groundwater environments.

Gen abstract type Poster

Section Type AEM

73 - Impacts of enhanced nitrogen fertilizer on soil microbial genes associated with plant growth promotion

Rebecca Pagès[1, 2], Mario Tenuta[2, 3], Matthew Bakker[1, 2]

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With the growing global population, fertilizers are critical to meet the rising demand for food. However, nitrogen (N)-fertilizer that is not assimilated by plants, whether due to NH_3 volatilization, NO_3^- leaching, or N_2O emissions, represents a loss to farmers and contributes to environmental degradation. To mitigate N losses, urease- and nitrification enzyme inhibitors are used by some farmers to slow nitrogen transformation rates and improve plant nitrogen uptake. However, we do not fully understand how these inhibitors impact soil microbial communities. We hypothesize that nitrogen-transformation inhibitors may impact microbial functional potential.

To explore this possibility, a field-based metagenomic study was conducted at the TGAS-MAN site for the long-term monitoring of greenhouse gas dynamics from agricultural soils (Glenlea, MB). Replicated wheat plots received either untreated urea (46-0-0), or an enhanced fertilizer formulation containing urea supplemented with the urease inhibitor N-(n-butyl)thiophosphoric triamide (NBPT) and the nitrification inhibitor dicyandiamide (DCD) (SuperU, Koch Agronomic Services). DNA was extracted from both rhizosphere and bulk soil samples and sequenced (Illumina NovaSeq X) to a median read depth of $1.19\times10^8 \pm 8.65\times10^6$ reads per sample. Data processing and analysis followed a standard pipeline of tools for quality control, trimming, *de novo* assembly, and functional annotation highlighting Plant Growth-Promoting Traits (PGPT), identified using the PGPg_finder workflow. From a median of $1.42\times10^7 \pm 1.50\times10^6$ predicted open reading frames (ORFs) per sample, $6.77\times10^6 \pm 8.27\times10^5$ were identified as PGPT-associated genes (corresponding to ≤ 6,910 unique PGPT-associated genes referenced in the PGPg_finder database). Differential abundance of PGPT-associated genes was assessed using linear models in the MaAsLin2 framework.

Differential abundance analysis revealed that the soil compartment (bulk soil vs. rhizosphere) was the primary driver of functional variation. The effect sizes (magnitude of response to soil compartment) of these PGPT-associated genes were highly correlated among fertilizer types (Spearman's $\rho = 0.977$, $p < 0.001$), with a 73.8% overlap in significant genes (2,266 genes significantly different between bulk soil vs. rhizosphere, under both fertilizer conditions). However, a compartment-specific analysis of the fertilizers type impact revealed a notable divergence: when assessing the fertilizer effect independently within each soil compartment, SuperU-fertilizer significantly altered the abundance of only 34 PGPT-associated genes in bulk soil, compared to 285 PGPT-associated genes in the rhizosphere. These findings suggest that SuperU-fertilizer exerts a disproportionately strong influence on microorganisms harboring PGPT-associated genes specifically within the rhizosphere. Interestingly, the distribution of genes encoding key nitrogen-cycling functions appears to be driven by compartment-specific factors rather than being uniform across the soil. Genes involved in nitrification, such as hydroxylamine oxidoreductase, were observed to be more abundant in bulk soil (Wilcoxon test, $p = 0.001$), whereas genes associated with urea uptake showed a trend toward enrichment in the rhizosphere (Wilcoxon test, $p = 0.052$).

These results demonstrate that the impact of SuperU-fertilizer is not intrinsic but is strictly context-dependent, varying fundamentally between bulk and rhizosphere soil. This observation aligns with PERMANOVA results, which confirmed that both fertilizer type and soil fraction significantly structure the genetic community, with a notable interaction effect (*adonis2*, model $p = 0.001$).

Section Type

Abstract selected for AEM Symposium June 19, 2026

283 - Understanding Physiological and Genotypic Diversity of *Pediococcus damnosus* in Spontaneous Beer Fermentations

Rylee Kager[1], Maanasa Mudoor Sooresh[2], Benjamin Willing[2], Benjamin Bourrie[1, 2]

[1]MacEwan University, [2]University of Alberta

Spontaneous beer fermentation is driven by complex natural microbial community assembly processes that are not directly controlled by brewers. Among these microbes, *Pediococcus damnosus* is one of the most prominent lactic acid bacteria (LAB), which significantly influence acidification, flavour development, and fermentation stability. Despite their prevalence, strain-level diversity regarding metabolic activity, exopolysaccharide (EPS) production and its functional consequences within the fermentation environment and product remain poorly characterized. This knowledge gap limits the ability to predict fermentation outcomes. Therefore, the objective of this project is to investigate how strain-level differences in *P. damnosus* influence EPS structure, composition, and fermentation performance in spontaneous beer.

Previous work isolated *P. damnosus* strains from four 6-month- to 2-year-old spontaneous fermentation barrels and confirmed their identification via 16S rRNA Sanger sequencing. Isolates were screened by RAPD-PCR, and eight were selected based on their DNA fingerprints. Of these, five were identified as having a “ropy” phenotype with mucoid colony morphologies and the remaining three were classified as “non-ropy.” In addition, isolates were grown on Congo Red MRS agar. Congo Red agar showed disparate results, with three of 5 “ropy” isolates producing strong positives, whereas two did not. Moreover, two of the three “non-ropy” isolates produced strong positive results, suggesting diversity in EPS composition among *P. damnosus* isolates.

Based on these initial findings, we hypothesized that genetically distinct *P. damnosus* strains produce structurally distinct EPS, leading to differences in fermentation dynamics and beer characteristics. Whole Genome Sequencing (WGS) of 4 representative isolates revealed the presence of core carbohydrate-active enzymes (CAZymes), including a broad range of glycosyltransferases. Interestingly, a putative signal peptide associated with the GH70 family of hexosyltransferases was identified in both ropy strains but not in the non-ropy strains. Future work will focus on characterizing the EPS structure to identify differences between the strains that may be important for fermentation dynamics and flavour development.

The results of this project will help clarify how genomic variation translates into fermentation outcomes and improve our understanding of the microbial contributions to fermentation dynamics. In addition, this project will identify candidate genes linked to variations in the *P. damnosus* population. Understanding functional diversity in spontaneous fermentations may support innovation in the brewing industry by enabling more predictable and customizable fermentation processes. Overall, this research contributes to the expanding literature on food-associated microbial ecosystems and highlights the importance of integrating ecology with microbiology. By emphasizing strain-level diversity within a bacterial species, this project challenges species-level generalizations used in fermentation studies. Ultimately, understanding how *P. damnosus* diversity influences spontaneous beer fermentation will enable more informed manipulation of abiotic conditions and starter culture composition to guide microbial communities and the resulting beer toward desirable, reproducible characteristics without compromising microbial diversity.

Section Type

Abstract selected for AEM Symposium June 19, 2026

166 - Characterizing Potential Antibiotic Resistance Genes (ARGs) in Polar Environments

Zhixuan Feng[1], Christina Davis[1], Jennifer Ronholm[1], Lyle Whyte[1]

[1]McGill University

Glacier shrinkage is a consequence of climate change and may expose microorganisms from a long-isolated environment to dynamic ecological systems. Recent studies have reported microbial activity in glacial meltwaters, raising concerns about the potential release and dissemination of antibiotic resistance genes (ARGs). ARGs naturally exist in bacteria living in harsh environments with limited nutrients, thereby increasing competitiveness. Although these microorganisms are unlikely to pose an immediate threat to human health due to their adaptation to extreme conditions (e.g., low temperature, hypersalinity, and anoxia), the possibility of horizontal gene transfer of ARGs into clinically relevant pathogens remains a public health concern. Understanding the diversity and distribution of ARGs in polar regions is therefore a critical first step in assessing this risk.

We hypothesized that Microbial communities in polar environments harbour a variety of ARGs and exhibit specific patterns that contribute to their survival, while posing a potential risk to public health. In this study, we aim to identify potential ARGs in the polar region, characterize their distribution, and collect these gene datasets for further tracing.

Metagenomic datasets from previous studies in Whyte Lab polar regions, including White Glacier, Johnsons Glacier, Gypsum Hill, and Lost Hammer, were analyzed using multiple ARG prediction approaches. Both alignment-based tools (ABRicate, RGI, AMRFinderPlus) and machine-learning-based tools (DeepARG and ARGNet) were used to predict ARGs. Redundant gene predictions across tools were removed, and only unique ARGs were retained for downstream analysis. In addition, samples from White Glacier were compared across different environmental contexts (glacial ice vs. forefield soil) to assess the potential impact of glacier retreat.

In the result, ARGs associated with multidrug resistance, aminoglycoside, glycopeptide, tetracycline, and macrolide-lincosamide-streptogramin (MLS) resistance were consistently detected across all samples. Also, in White Glacier, ARG composition and diversity differed markedly between glacial ice and forefield soil samples. Overall, ARG diversity was substantially higher in soil samples than in ice samples. Multidrug resistance genes exhibited the highest diversity in both environments, while genes associated with glycopeptide, aminoglycoside, and diaminopyrimidine resistance were more diverse in soil than in ice.

This study provides an ARGs profile in diverse polar environments using multiple detection tools. The results suggest that polar microbiomes harbour a diverse reservoir of ARGs, with diversity potentially increasing following glacier retreat. Despite this, the overall risk to human health would be limited due to low microbial abundance and environmental constraints. Nevertheless, the potential for ARGs functional expression in the microorganism and dissemination via horizontal gene transfer warrants further investigation.

Section Type

Abstract selected for AEM Symposium June 19, 2026

182 - Diverse phages of ammonia oxidizers with the potential to modulate nitrification

Aaron Turner[1], Miranda Stahn[2], Andrew Millard[3], Dominic Sauvageau[2], Lisa Stein[1]

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Agriculture is a major source of anthropogenic greenhouse-gas emissions and the largest source of nitrous oxide (N_2O), a potent greenhouse gas and ozone-depleting agent. Soil N_2O emissions are largely driven by microbial nitrification, in which ammonia-oxidizing microorganisms catalyze the rate-limiting oxidation of ammonia to nitrite. Nitrification not only mediates N_2O fluxes but also reduces fertilization efficiency and contributes to eutrophication through nitrate leaching. Bacteriophage (phage)-based control of microbial communities is rapidly garnering interest; however, phages infecting ammonia-oxidizers are largely under-researched, with only one lytic phage having been described, limiting the potential for phage-mediated nitrification inhibition. Here, we characterized a large set of phages infecting ammonia-oxidizing bacteria (AOB): 45 dsDNA phages identified from urban wastewater, infecting four AOB species, with 10 demonstrating cross-genus host ranges and capable of eliminating nitrification activity in liquid cultures. Phylogenetic and taxonomic analyses revealed six proposed families of *Caudoviricetes* and numerous monophyletic clades, likely representing higher-level lineages. Structure-guided genome annotation revealed that these phages carry diverse and seldom-seen auxiliary metabolic genes, including a complete ABC transporter cassette and a complete cytochrome bc1 complex. These results unveil previously unrecognized diversity, broad taxonomic and functional repertoire, and the potential of AOB phages to alter host physiology. Moreover, we outline an effective methodological framework for isolating low-abundance phages targeting recalcitrant hosts. These results reframe our understanding of nitrification and the microbes that mediate it, and enable intensified selection and use of phages for its control.

Section Type

Abstract selected for AEM Symposium June 19, 2026

229 - Organic acids and bacteriophages as alternative to antibiotics for the poultry industry

Alba Park de la Torriente[1], Brandon Spoja[1,2], Alireza Amanatchi[1], Gabhan Chalmers[1], Maysa Niazy[3], Kimia Mehrafshan[1], Ayesha Lone[2], Hany Anany[2], Nicole Ricker[1]

[1]Pathobiology, OVC, University of Guelph, [2]Guelph Research and Development Centre, AAFC, [3]McMaster Immunology Research Centre, Department of Medicine, McMaster University

With the rise of antimicrobial resistance as a major worldwide concern, and the implementation of regulatory restrictions on the agricultural use of medically important antimicrobials in Canada, the search for alternative strategies to enhance productivity and prevent/control infectious disease outbreaks in the poultry industry has become paramount. Different interventions—such as feed and water additives, probiotics, vaccines and bacteriophages—have been used by poultry farmers, hatchers and growers. Most of these interventions have not proved to be consistently effective on their own, but a combination of strategies may yield better results.

We aim to evaluate organic acids, bacteriophages and the combination of both as a strategy to enhance productivity and control infectious diseases in the poultry industry —with a particular focus on *Salmonella*. Dietary organic acids have shown a positive effect in productivity parameters by directly eliminating pathogens and other non-acid tolerant bacteria, or by stimulating competitive exclusion of pathogens. Bacteriophages, on the other hand, provide an opportunity for reducing the burden of specific pathogens without disrupting the gut microbiome of the hosts.

In the first stage of this project, we investigated the effects of organic acids during the transition to omega-3 feed in layers. For this, a longitudinal field study was conducted in collaboration with a commercial egg producer in which the hens were given a blend of organic acids in the drinking water before the transition to the omega-3 diet. We found that a decline in feed consumption and egg production could be prevented with the additive, as well as a reduction in illness-associated mortality. Although the addition of the organic acid significantly decreased taxa diversity, microbiome analysis demonstrated this was restored after the transition to the omega diet, despite continued use of the organic acid. Observed changes in the microbiome indicated that the addition of organic acid during the transition to the omega-3 diet supported a healthy gut environment. Furthermore, the abundance of potentially pathogenic and opportunistic genera—such as *Clostridium*, *Escherichia*, *Enterococcus*, *Gallibacterium* and *Staphylococcus*—was significantly decreased with the dietary interventions.

The subsequent part of the project aims to explore the potential of bacteriophages and organic acids as pre-harvest *Salmonella* control measures. For this, we selected a phage combination that can cover a large proportion of *Salmonella* serovars based on an infection screen. After characterizing the phage cocktail, we began studying the effects of the phage cocktail, the acidification and their combination on a simulated chicken gut microbiome. In this pilot study, we used a closed system that combined a bile salt media and ileum contents to create a realistic and complex environment. Preliminary results indicate that 1) the phages are tolerant to the acid in this complex environment, and 2) uninhibited XLT-4 gram-negative bacteria (likely *Enterobacter spp., Citrobacter spp.* and *Escherichia coli)* are significantly reduced with the acidification treatment. Salmonella enumeration by qPCR is pending. Further studies include the optimization and biological replicates of the gut model assays, followed by a live animal trial.

Section Type

Abstract selected for AEM Symposium June 19, 2026

337 - Impact of Strain and Isolation Method on Outer Membrane Vesicle Yield and Morphology in *E. coli*

Jack Vallis[1], Geneviève Loiselle[1], Shelby Reimer[1], Daniel Beniac[2], Shannon Hiebert[2], Timothy Booth[1, 2], Patrick Chong[2], Garrett Westmacott[2], George Zhanel[1], Denice Bay[1]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada, [2]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada

Introduction Bacteria secrete extracellular vesicles to communicate with other cells, evade host defences, and acquire nutrients. Bacterial outer membrane vesicles (OMVs) are produced by Gram-negative bacteria and comprise the cell's lipid bilayer, ranging in size from 20-400 nm in diameter. OMVs are released from the outer membrane (OM) and possess all the same biological properties of the cellular OM as their originating cell. OMVs are natural delivery vehicles used to transfer intracellular content between similar and selective bacteria. They deliver virulence factors and confer antibiotic-resistance enzymes on nearby bacteria, protecting their interior cargo from extracellular degradation.

Gram-negative bacteria are resistant to many antibiotics due to their dual cell envelope. An advantage of using OMVs for drug delivery is their extreme stability when compared to artificial lipids and their ability to protect their content from enzymatic degradation. The ability of OMVs to deliver molecules as cargo has led to their increased use and the development of drug-delivery vehicles for cancer and other hard-to-deliver targets. One application of OMVs as a drug-delivery tool may be to re-engineer them to thwart antimicrobial resistance (AMR). However, OMV production by wild-type (WT) *E. coli* is typically low, limiting their scalability and practical use. Comparing strategies to enhance OMV yield, including the use of hypervesiculating mutants and different isolation methods, is critical for advancing OMV-based technologies.

Methods WT *E. coli* K-12 and hypervesiculating *tolA* and *nlpI* deletion mutants were cultured under identical growth conditions, and OMVs from each strain were isolated via ultracentrifugation and either stirred cell (SC) ultradiafiltration or Tangential Flow Filtration (TFF). Tricine SDS-PAGE was performed to separate individual OMV proteins to determine if there were any differences in protein content between OMVs. Nanoparticle Tracking Analysis (NTA) and cryogenic transmission electron microscopy (cryo-TEM) characterized particle yield, size and morphology.

Results While the isolation time for WT OMVs was not significantly different between SC and TFF ($p=0.91$), TFF significantly reduced the time required to isolate hypervesiculating mutant vesicles ($p=0.01$). TFF also resulted in a $\log_{10}$ fold increase in WT-OMV yields, while no significant differences were noted between SC and TFF OMV yields in *tolA* deletion mutants ($p=0.81$). Both isolation methods produced OMVs of similar diameters. SDS-PAGE showed no obvious differences in OMV banding patterns for either WT-OMVs or OMVs from *tolA* deletion mutants recovered by SC or TFF. Based on cryo-TEM images, nearly all WT-OMVs had a single membrane, whereas a high proportion of OMVs from *tolA* deletion mutants comprised two or more membranes (42.4%). When using TFF as an isolation method, *tolA* deletion mutants produced significantly more OMVs than WT *E. coli* and *nlpI* deletion mutants.

Conclusions These findings show that OMV yield and morphology are strongly influenced by both bacterial strain and isolation methods. The enhanced OMV production observed in *tolA* deletion mutants and TFF methods supports their use in optimizing OMV isolation. Ongoing work is extending these findings to additional hypervesiculating mutants, including *nlpI* deletion mutants, to further improve strategies for OMV drug-delivery applications.

Section Type AEM

Abstract selected for AEM symposium June 19, 2026

Workshops and Panelists/ Ateliers et intervenants

198 - Too much of a good thing: FtsK disassembly is critical for functional bacterial cell division in *Escherichia coli*

Cezar Khursigara[1]

[1]University of Guelph

The essential *Escherichia coli* cell division protein FtsK is a leading candidate for the long-sought divisome checkpoint that couples chromosome segregation to septation. Yet the only portion of FtsK required for division, its N-terminal membrane-integral domain, $FtsK_N$, has resisted conventional structural analysis for three decades, leaving its mechanism a persistent gap in the field of cell division. Here, we close that gap by combining coevolutionary contact prediction, AlphaFold modelling, and targeted mutagenesis to probe the structure of $FtsK_N$ directly in its native membrane, and we uncover a counterintuitive rule governing its function.

Using coevolutionary analysis, we identified pairs of putatively interacting residues across $FtsK_N$ and designed reciprocal break-and-restore mutations at each predicted contact. Plasmid-borne $FtsK_N^{220}$ variants were expressed in the temperature-sensitive *ftsK44* strain LP11-1 at the non-permissive temperature (42 °C), and function was quantified by cell-length analysis. Restore mutations rescued the division defect for a remarkable number of break pairs, validating the workflow and partitioning the contacts into three mechanistically distinct classes: Group 1 pairs that confirm the AlphaFold monomeric fold; Group 2 pairs that lie at distances incompatible with the static model and implicate conformational flexibility in the periplasmic loops; and, most unexpectedly, Group 3 pairs separated by distances reconcilable only by lateral contacts between adjacent monomers along helices α1 and α6 — direct *in vivo* evidence of an oligomeric interface. AlphaFold multimer modelling of these interfaces converges on a modular lateral assembly rather than the rigid hexameric ring formed by $FtsK_C$.

We then asked the central mechanistic question: does breaking oligomerization break function? Immunofluorescence microscopy showed that Group 3 break mutants localize normally to mid-cell despite their division defect, uncoupling oligomerization from recruitment. Preliminary SEC-MALS analysis then delivered the counterintuitive punchline: the L32F break mutation does not abolish the hexamer, it enriches it, shifting the monomer-to-hexamer equilibrium toward the assembled state. Function is lost not because $FtsK_N$ fails to oligomerize, but because it fails to disassemble.

These findings invert the expectation for an oligomeric cell division protein and establish timely disassembly as a candidate requirement for $FtsK_N$ function. Defining how oligomer dynamics are coupled to septal progression, and whether this coupling underlies FtsK's proposed checkpoint activity, is now experimentally tractable through the mutations and framework described here.

Section Type MGCM

Invited Speaker Panel Discussion: AI in publishing June 18, 2026

416 - Identifying Genes Important for Salmonella Survival in Water

Rylan Bahrey[1, 2], Sylvia Li[1], Samantha Jelfimow[1, 2], Michelle Gerber[1, 2], Jessica Sparrow[1], Young Min Kwon[3], Mohamed Helmy[1], Aaron White[1, 2]

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Salmonella spp. are a major causative agent of human diarrheal disease worldwide, with up to a 30% mortality rate in developing countries. Despite this, researchers still do not understand the specifics of how Salmonella spp. are able to survive outside of a host. Survival in water and subsequent transmission are thought to be important for pathogenic success, but there is a gap in knowledge on how Salmonella can do this. Salmonella uses biofilms to survive desiccation, resist antimicrobials and increase survivability in general, therefore it has been hypothesized that Salmonella would use its biofilm capabilities to survive in water. To answer the question of what genes are important for Salmonella to survive in water, we built a transposon (Tn) library, where each strain contains a single Tn insertion, leading to inactivation of a gene. The entire collection of strains in the library represents every gene in the Salmonella genome. By inoculating the Salmonella Tn library into water and incubating for 50 days, we can determine what genes are most important for water survival. By comparing the Tn-library after 50 days in water (the output) to the starting Tn-library (the input) we are able to tell what genes are important. Through multiple experiments and replicates, we can differentiate between genes that are consistently identified versus those that appear randomly. I will describe the process of building and validating the Tn library and show data from our water survival experiments, including some of the first genes we have identified. Using a Tn library to investigate water survival will lead to a greater understanding of the mechanisms used by Salmonella to transmit and cause new infections.

Abstract category choice

Invited Speaker & Exhibitor (reserved only for those receiving specific invitations)

Section Type

MGCM

ABSTRACTS ONLY/ RESUMES UNIQUEMENT

228 - Molecular choreography of phage defence: sensing viral assembly to abort infection

Karen Maxwell[1]

[1]University of Toronto

The viruses that infect bacteria, known as bacteriophages or phages, have evolved alongside a vast repertoire of bacterial defence systems that collectively define the microbial immune landscape. The enormous genetic diversity presented by phages raises a central question of how these systems detect the presence of an infecting phage. We discovered a class of bacterial defence proteins that sense the molecular choreography of viral assembly, detecting oligomeric intermediates of phage structural proteins to trigger abortive infection. Using biochemical, structural, and genetic approaches, we show that this mechanism couples real-time surveillance of phage virion assembly with rapid host self-sacrifice, effectively halting viral propagation. This work expands the conceptual boundaries of antiphage defence recognition and highlights evolutionary convergence between bacterial and eukaryotic immune strategies that detect the molecular signatures of infection.

Gen abstract type

Abstract only

Section Type

AEM

287 - Exploiting bacteriophage functionalities to engineer microbial cells to act as live cell therapeutics

David Stuart[1], Laura Enekegho[2], Karla Cruz[2]

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The ominous rise in the frequency of antimicrobial resistant (AMR) bacterial infections poses a global threat to human health. This threat comes in the direct form of disease related to infection of humans but also indirectly in the form of loss of food security through loss of agricultural crops and animals to disease. Food-borne pathogens are responsible for a considerable portion of the illness and burden imposed on the healthcare system and are responsible for substantial economic loss imposed on the agricultural food production systems. Among the most problematic food-borne pathogens, the gram-positive bacterium *Clostridium perfringens* is an opportunistic pathogen capable of causing necrotic enteritis upon intestinal infection and gas gangrene with tissue necrosis in infected wounds. Both human populations and agricultural animals are affected by *C. perfringens* infection. The development of antibiotic drugs in previous decades was transformational, providing cheap, effective treatment for what would otherwise would be a lethal infection. Antibiotic drugs are the standard treatment and prophylactic antibiotics have been used by poultry farms to limit clinical and subclinical infection of animals. As *C. perfringens* strains resistant to front-line antibiotic drugs have emerged, there is an urgent need to develop new therapeutic strategies and tools to control infections. Synthetic biology and metabolic engineering can be applied to generate "weaponized" live cell therapeutics providing new means to combat the rapidly evolving threat of microbial pathogens. We have engineered probiotic yeast to produce and secrete bacteriophage derived endolysins capable of lysing *C. perfringens* cells. A series of signal peptides were tested to identify an optimal secretion signal. Subsequently, a combinatorial engineering approach was taken to optimize the probiotic cells secretory pathway. The engineered strain yielded a near 20-fold increase in endolysin secretion. Turbidity and halo assays demonstrate effective killing of pathogenic *C. perfringens* with little to no lysis of lactobacillus strains native to the human and poultry gut. Endolysin secretion was also demonstrated in intestine like fluid medium confirming that the probiotic can produce endolysin under conditions that mimic the gut environment. To improve specific targeting of *C. perfringens* nanobodies and peptides with high affinity for the *C. perfringens* cell wall were displayed on the surface of the probiotic yeast endowing it with the capability of binding to *C. perfringens* cells and colonies. The live cell therapeutic platform has been further exploited to secrete nanobodies capable of neutralizing bacterial toxins into the gut to reduce tissue damage caused by pathogen infection. We demonstrate that engineered probiotics display potential to control AMR pathogens and inhibit toxins they secrete in the gut and likely anywhere that they can be delivered.

Gen abstract type

Abstract only

Section Type

AEM

280 - Evolution system selection influences the evolution of antimicrobial resistance in *E. coli*

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Adaptive laboratory evolution systems (ALEs) are frequently used to estimate the likelihood of antibiotic resistance and predict resistance mechanisms before they can arise in the clinic. Unfortunately, the lab environment is not the clinic, and variations in selective pressure can change evolutionary outcomes in unanticipated ways.

We have found for the first time that the type of laboratory evolution system itself changes the selective pressure cells experience, with significant differences in evolutionary pathways. Specifically, we have compared liquid serial passage, streaking on agar, and soft agar gradient evolution, evolving *Escherichia coli* BW25113 against tigecycline and piperacillin (n = 20-24 for each drug/ALE pair).

Of the three techniques tested, SAGE best matched resistance patterns from a panel of over 750 pathogenic *E. coli* isolates. In general, we found that cells which evolved antimicrobial resistance through serial passage in liquid or on agar more frequently developed collateral sensitivity (an increase in overall susceptibility) to antibiotics with unrelated mechanisms, while cross-resistance was far more common in both clinical isolates and cells that evolved via SAGE. Serial passage in liquid media is one of the most common ALEs, and as a result the current literature may over-estimate the frequency of collateral sensitivity.

This work shows that pitfalls remain prevalent in the design of adaptive laboratory experiments, and there is a strong need to closely match outcomes to clinical data and the natural environment cells encounter.

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380 - Phospholipid composition alterations change integron-associated efflux pump drug selectivity and lipid-protein co-associations in *Escherichia coli*

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Integron-transmitted efflux pumps can recognize and expel a broad and overlapping range of antimicrobials (antibiotics and antiseptics); often favoring cationic and lipophilic substrates. Pump substrate selectivity is typically considered at the sequence or folded protein structure level, however, when carried on integrons, these proteins must properly fold and adapt to different membrane phospholipid (PL) compositions to expel drugs effectively. The interactions between efflux pumps and their stabilizing annular PLs is unclear; protein-PL perturbations may also represent novel antimicrobial targets to inhibit or modulate efflux pump activity.

This study assessed *E. coli* K-12 strain PL biosynthesis mutants for their impacts on the antimicrobial susceptibilities of four cation-selective, integron-associated Gram-negative efflux pumps (EmrE, MdfA, MdtK, and AceI). Each pump was expressed in a mutant unable to biosynthesize one of the three major PLs: phosphatidylethanolamine (PE; Δ*pssA*), cardiolipin (CL; Δ*clsABC*), or phosphatidylglycerol (PG) and CL (Δ*pgsA* + *lpp-2*). Antimicrobial susceptibility testing revealed that the loss of PE resulted in the most significant reductions (>4-fold) to minimum inhibitory concentration (MIC) values by all 4 pumps. Losses of CL or CL+PG by mutants expressing MdfA or AceI resulted in broader MIC profile shifts across multiple antiseptic/antibiotic classes tested. Annular PLs that co-associated with each peptidisc solubilized His_6-affinity tagged efflux protein were identified by electrospray ionization mass spectrometry. This analysis showed that all 4 efflux proteins had an overwhelming bias towards CL and lysoCL lipids (70-95% of total PLs), where only 45-55% of these CLs were identical, but each pump protein had unique ratios of CL, lysoCL, PE, PG, and lysoPG.

This study shows that PL composition impacts efflux activity and substrate recognition. This may explain why integron-associated efflux activity varies across different species with unique PL contents.

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