# Invited Keynote Speakers & Session Speakers Abstract

## SESSION I: Viruses: From Environments to Clinics
## Tuesday, June 19th, 2018
## 8:30 - 10:00 AM

**IS 02**
**Towards a Universal Influenza Virus Vaccine**
Peter PALESE, Icahn School of Medicine at Mount Sinai

Despite FDA-approved vaccines and antivirals, seasonal and pandemic influenza remain serious threats associated with substantial morbidity and mortality. While annual seasonal influenza virus vaccination is frequently effective – albeit underutilized in most countries – a safe universal influenza virus vaccine providing broad and long-lasting immunity would represent a major breakthrough. We have developed vaccine constructs which express chimeric hemagglutinins resulting in the redirection of the immune response away from the immunodominant (variable) head domain of the hemagglutinin towards the much more conserved stalk of the hemagglutinin and the highly conserved neuraminidase. Such vaccine constructs work well in animal challenge models and are currently tested in clinical trials in humans. The mechanism by which these novel vaccines mediate protection is via antibodies which do not rely on hemagglutination inhibitory (HI) activity but rather on ADCC (antibody-dependent cell-mediated cytotoxicity) effects, activation of complement and/or inhibition of virus replication through directly binding to viral proteins. It is hoped that the universal influenza virus vaccine based on chimeric hemagglutinins will provide long-lasting protection against all seasonal and pandemic influenza virus strains in the future.

**SS1-1**
**Potent inhibition of pathogenic viruses using ubiquitin variants that target viral deubiquitinases**
Brian MARK, University of Manitoba, W. ZHANG[2], B.A. BAILEY-ELKIN[1], R.C.M. KNAAP[3], B. KHARE[1], T.J. DALEBOUT[3], G.G. JOHNSON[1], P.B. VAN KASTEREN[3], N.J. MCLEISH[1], M. KIKKERT[3], S.S. SIDHU[2], [1]University of Manitoba, [2]University of Toronto, [3]Leiden University

Post-translational modification of cellular proteins by ubiquitin regulates numerous cellular processes, including innate and adaptive immune responses. Ubiquitin-mediated control over these processes can be reversed by cellular deubiquitinating (DUB) proteases, which remove ubiquitin from cellular targets and depolymerize polyubiquitin chains. The importance of protein ubiquitination to host immunity has been underscored by the discovery of viruses that encode proteases with DUB activity, many of which actively corrupt the cellular ubiquitin system to suppress innate antiviral responses and participate directly in viral replication processes. DUB enzymes have now been identified in diverse viral lineages and their characterization is providing valuable insights into virus biology and the role of the ubiquitin system in host antiviral mechanisms. Recently, screening a phage-displayed library of billions of Ub variants (UbVs) we identified UbVs that are potent inhibitors of DUB proteases from the *Nairovirus* Crimean Congo Hemorrhagic fever virus (CCHFv) and the *Nidovirus* Middle East respiratory syndrome coronavirus (MERS-CoV). While maintaining the structural scaffold of wild type Ub, the UbVs contain specific amino acid substitutions that greatly enhance the binding affinity toward the cognate DUB enzymes. In particular, the UbVs specific for the MERS-CoV protease (PLpro) potently and specifically inhibited DUB activity and the viral polyprotein processing activity of the enzyme. The molecular basis for this increased affinity was elucidated by X-ray crystallography. UbVs bound to PLproin an identical orientation to wild type Ub, and several residues unique to the UbVs formed strong hydrophobic and hydrogen bonding interactions with PLpro. Remarkably, expression of the most potent UbV in MERS-CoV-infected cells resulted in a greater than 4-log reduction in progeny virus titers, demonstrating the therapeutic potential of MERS-CoV-specific UbVs.

**SS1-2**

**Navigating the Storm: merging basic research with clinical information for (re)emerging infectious diseases**

Jason KINDRACHUK, University of Manitoba

Emerging and re-emerging viruses pose a significant threat to global public health. Outbreaks attributable to these pathogens, including ebolaviruses and influenza viruses, continue to increase in frequency as a result of changing socio-economic, environmental, and ecological factors. Many of these viruses result in severe illness and complex pathogenesis during the course of infection; however, the molecular processes underlying these events are often poorly understood. To this end, an integration of basic and clinical research efforts can accelerate translational research for emerging and re-emerging viruses. Detailed molecular investigations of the severe clinical and pathologic manifestations associated with these viruses provides important insight into disease pathogenesis and may advance therapeutic discovery. OMICS-based methodologies that investigate global changes in host cellular responses provide the opportunity to characterize molecular events related to pathogenesis and facilitate therapeutic target identification. Characterization of the global activation state of host cell kinases (the kinome) provides direct insight into cellular responses at the level of complex cell signaling networks and individual kinases.Kinome analysis provides critical insight into viral pathogenesis and dysregulated cell responses.Further, it can also facilitate biomarker discovery and drug, or drug target, identification. The utility of kinome analysis and novel basic research approaches for investigating emerging and re-emerging viral diseases will be described with a particular focus on pathogenesis at blood-tissue barriers. Importantly, recent examples from my laboratory will highlight: i) the relation of viral-mediated cell response dysregulation and disease severity; and ii) characterization of molecular pathogenesis during the acute and recovery phases of severe viral disease.

**SS1-3**

**Post-Exposure Administration of Whole Inactivated H5N1 Influenza Vaccine Protects Mice From Lethal Homologous Infection.**

Darwyn KOBASA, Public Health Agency of Canada, J AUDET[1], C RANADHEERA[1], J MORIN[2], A LEUNG[1], M CHAN[1], [1]Public Health Agency of Canada, [2]Sherbrooke University

Objectives: Highly pathogenic avian influenza viruses, such as H5N1, are found throughout wild aquatic birds and domestic poultry, with certain strains that are able to cause significant disease in humans. Although these avian influenza viruses do not readily infect humans, their ability to mutate quickly and reassort genes with other influenza strains may allow these viruses to adapt to better infect humans. Recently, H5 viruses originating from Eurasia have appeared for the first time in North American wild and domestic bird populations. The wide range of H5 subtypes circulating globally makes it impractical to pre-vaccinate all individuals at high risk of exposure to these viruses. Instead, we were interested in evaluating the efficacy of a formalin-inactivated influenza virus, analogous to the conventional influenza vaccine, as a post-exposure vaccine therapy. **Methods:** We used a well characterized strain of H5N1, A/Vietnam/1203/2004, to generate a whole formalin-inactivated influenza vaccine for post-exposure treatment. Mice were infected with a lethal dose of H5N1 and treated with the whole inactivated vaccine up to 3 days post-infection. Protection was assessed based on weight loss and severity of clinical signs and symptoms. Levels of CD4+ and CD8+ T cell responses were measured by flow cytometry, while antibody responses such as total IgG, IgM, and IgA were determined by ELISA. Microneutralization assays were used to measure levels of neutralizing antibodies in the serum. **Results:** Immediate post-exposure treatment with a whole inactivated H5N1 vaccine afforded complete protection from lethal outcome following homologous virus infection. A delay in treatment up to 24h post-exposure remained highly efficacious, however, treatment after 24h post-exposure was no longer protective. By measurement of T cell and antibody responses, we show that whole inactivated H5N1 rapidly induced a protective immune response in mice after the infection. In vaccine treated mice, protection was associated with the generation of a more rapid and robust antibody response. We also observed that vaccination resulted in decreased activation of CD8+ T cells, suggesting a dampening of the Th1 response that is generally associated with pro-inflammatory immune responses, and may have contributed to the recovery of all mice that were treated at the optimal dose of vaccine. **Conclusion**: These results offer a new strategy of using inactivated vaccines as an effective post-exposure treatment against H5N1 viruses, and present another option to provide protection for front-line responders in the event that a new pandemic strain emerges.

## SESSION II: Genomics and Bioinformatics
## Tuesday, June 19th, 2018
## 8:30 - 10:00 AM

**IS 03**
**Evolutionary and Temporal Views of Microbial Diversity**
Robert BEIKO, Dalhousie University

The microbiome is a mess – hundreds of types of microorganisms, each with thousands of genes, all performing a different set of tasks. To have any hope of understanding how the microbiome works, and how it drives and is driven by changes in the surrounding habitat, we must be able to define what exactly is meant by "types". This is not a trivial task, and many imperfect methods, some more imperfect than others, have been introduced to try and delineate different actors in the microbiome. For example, one can survey markers such as the 16S ribosomal DNA gene and cluster them based on some uniform or flexible similarity criterion, or do a metagenomic survey and try to reconstruct genomes. However, ecological (mis)behaviour does not correlate perfectly with sequence similarity, and we need to consider flexible approaches that can move beyond sequence similarity alone. In my talk I will share some ideas about how to complement sequence similarity using other approaches, and illustrate the performance of some of these on interesting test data sets. For example, we have used phylogenetic and functional approaches to construct alternative views of the microbiome, and used machine learning to assess the effectiveness of these representations relative to naïve similarity-based cutoffs. Our results demonstrate that considering phylogenetic clades at different degrees of similarity can improve our ability to distinguish different habitat types. We have also developed a new tool, Ananke, that expresses similarity in terms of temporal profiles rather than sequence similarity, and uses graph-clustering approaches to identify subsets of organisms that covary through time. Not surprisingly, these clusters often map well onto sequence-similarity profiles, but there are interesting exceptions, including many examples in which very similar sequences are assigned to clusters with very different temporal profiles. Indeed we often see examples of "stealth" swaps where one marker-gene sequence is completely replaced by a nearly identical one. It would be a mistake to completely abandon sequence similarity as a criterion for defining "types" within a microbiome sample, but our results to date clearly demonstrate that these signals need to be augmented by other views of the information we collect from microbiome samples. These and other ideas will allow us to build better pictures of microbiome diversity and function.

**AEM 092**
**GenOme Resolution by Density-gradient Isopycnic ANalysis (GORDIAN) for sequence-based microbial community analysis**
NP BULLEN, University of Waterloo, S COYOTZI[1], S THIJS[2], J VANGRONSVELD[2], WE HOLBEN[3], LA HUG[1], Josh NEUFELD[1], [1]University of Waterloo, [2]Hasselt University, [3]University of Montana

Despite the advent of cultivation-independent methodologies for microbial community analysis, comprehensive characterization of microbial communities remains a challenge due to the magnitude of diversity that exists in most environments. A major challenge is that modern high-throughput sequencing applied to 16S rRNA gene amplicons or shotgun metagenomes results in high coverage of templates from abundant taxa, yet only low access to extensive diversity that exists at lower relative abundance. In order to circumvent this challenge, we adopted a previously developed "GC fractionation" approach for use with high-throughput sequencing, which together we refer to as GenOme Resolution by Density-gradient Isopycnic ANalysis (GORDIAN). Using bis-benzimide to accentuate GC:AT ratio-specific density differences, isopycnic cesium chloride density gradients separated microbial community DNA into distinct fractions based on genomic guanine and cytosine (GC) content. We pioneered GORDIAN for comprehensive analysis of DNA extracted from soil (agricultural and forest; high diversity), a wastewater treatment facility (rotating biological contactor biofilm; medium diversity), and a defined community (five pure bacterial cultures; low diversity). All fractions were characterized by 16S rRNA gene sequencing on a MiSeq (Illumina) prior to shotgun metagenomic sequencing. The 16S rRNA gene results demonstrated strong separation of genomic DNA, based on distinct taxonomic profiles associated with the fractions from each gradient. Comparison of 16S rRNA gene profiles to reference genomes with known GC content confirms that GC content governs fraction distributions. Individual fractions represent a subset of the diversity detected in the unfractionated sample, and many relatively rare taxa from

the original unfractionated samples dominated fractions associated with high and low GC contents. In particular, operational taxonomic units (OTUs) associated with *Thaumarchaeota*, *Nitrospira*, and *Bacteroidetes* dominated individual fractions by ~10-fold greater relative abundance compared to the unfractionated sample. Several low abundance OTUs were detected in fractionated sample data that were undetected in the unfractionated sample, including those affiliated with uncultivated bacteria from diverse higher lineages and candidate genera such as *Candidatus* Nitrosocosmicus from the *Thaumarchaeota*(relatively low GC content). Overall, these 16S rRNA gene results demonstrate the promise of applying GORDIAN for accessing unique microbial populations with complex microbial communities with shotgun metagenomics, which is ongoing for these same samples.

**SS2-2**

**How many SNPs are enough SNPs? Correctly Identifing Colonal Isolates, Using Whole Genome Sequencing, During a Foodborne Outbreak Investigation**

NP NICHOLAS PETRONELLA, Health Canada, PK PALINI KUNDRA[2], SB SWAPAN BANERJEE[1], AG ALEX GILL[1], FP FRANCO PAGOTTO[1], ST SANDEEP TAMBER[1], KK KYLE KINGSLEY[3], Jennifer RONHOLM[2], [1]Health Canada, [2]McGill University, Macdonald Campus, [3]Applied Maths, Inc.

Whole Genome Sequencing (WGS) is rapidly replacing other molecular techniques for identifying and sub-typing bacterial isolates during foodborne outbreak investigations. Ultimately, delineating a foodborne outbreak hinges on correctly determining if bacteria isolated from patients, food-samples, and food-processing plants are clonal; in other words, did they originate from a single source? Accurate source attribution is the basis of food recalls. Therefore, techniques are required that can sub-type bacterial isolates at a high-enough resolution to differentiate strains that are closely related from strains that are truly clonal. WGS followed by comparison between isolates at the nucleotide level is the highest resolution method for sub-typing isolates since it makes use of the entire genome. Techniques like multi-locus sequence typing (MLST) or pulsed field gel electrophoresis (PFGE) only utilize small subsections of the genome. Once the genomes of isolates suspected to be from the same outbreak are whole genome sequenced they must be compared to find nucleotide differences, called single nucleotide polymorphisms (SNPs). An important question from both a scientific and regulatory perspective is: How many SNPs have to be found to conclude that two genomes are unrelated? In this study, we selected four Shiga toxin producing *Escherichia coli*, four *Salmonella enterica*, three *Listeria monocytogenes*, and two *Vibrio parahaemolyticus* isolates. We sub-cultured each of these thirteen isolates one-hundred times. We whole genome sequenced the first subculture and the last subculture and then compared the sequences using a variety of SNP-calling bioinformatics pipelines including: a reference guided approach (SNVPhyl), whole genome multi-locus sequencing typing (wgMLST), and a whole genome SNP calling (wgSNP) approach. The number of SNPs generated during one-hundred sub-cultures varied greatly between bacterial species. To illustrate this point, using the wgSNP technique, one *E. coli* isolate had 310 SNPs, while one *L. monocytogenes* isolate had no SNPs between the two cultures. Not surprisingly, the number of SNPs also varied greatly dependent on the calling method used. SNVPhyl called the fewest SNPs and wgSNP called the most. This study provides a baseline for the number of SNPs expected during bacterial growth over a time frame that is relevant to foodborne outbreak investigation for four important foodborne bacterial pathogens. We also call attention to the need for a standardized SNP-calling pipeline to be used for outbreak investigations.

**II 078**

**Quantitative Proteomics of the Host-Pathogen Interplay during *Salmonella* Typhimurium Infection**

Jennifer GEDDES-MCALISTER, Max Planck Institute of Biochemistry, S VOGT[2], J ROWLAND[2], S WOODWARD[2], B FINLAY[2], F MEISSNER[1], [1]Max Planck Institute of Biochemistry, [2]University of British Columbia

Intracellular bacterial pathogens are capable of causing a diverse array of diseases in humans and represent a significant threat to global health. These pathogens have evolved sophisticated strategies including the secretion of virulence factors to interfere with host cell functions and to perturb immune responses. However, interplay between the host and pathogen at the protein level in the context of infection has not been systematically investigated. Our 'infectome' analysis aims at the identification of previously undescribed proteins involved in bacterial virulence as well as host immune defense, representing an opportunity to elucidate molecular mechanisms of host-pathogen interplay during disease. Here, we investigate the host-pathogen interplay between the pathogenic bacteria, *Salmonella* Typhimurium, and bone-marrow-derived macrophages. We performed quantitative proteomics of the host cells infected with wild-type (SL1344) or the type 3 secretion system (T3SS) mutant (dspi-1 and dspi-2) strains in single runs using high resolution mass spectrometry on a Quadrupole Orbitrap instrument. Overall, we provide a comprehensive and dynamic view of both pathogen and host proteins during infection. In the host cells, we observed the upregulation of proinflammatory and lysosomal proteins, representing host defense mechanisms to initiate immune responses and combat bacterial invasion. For *S.* Typhimurium, integration of proteome and infectome data identified proteins not encoded on the T3SS pathogenicity islands as being co-regulated with known virulence factors, suggesting a co-functional role in virulence and infection. Current work is pursuing the mechanistic characterization of Spi1- and Spi2-associated candidate pathogenic proteins. Overall, our approach represents an innovative strategy to comprehensively characterize and elucidate molecular mechanisms of host-pathogen interplay during disease from both host- and pathogen-centric perspectives.

## SESSION III: Bacterial Stress Response
## Tuesday, June 19th, 2018
## 8:30 - 10:00 AM

**IS 04**

**A high-frequency phenotypic switch regulating virulence in *Acinetobacter baumannii***

Philip RATHER, Emory University

*Acinetobacter baumannii* strain AB5075 can rapidly switch between two phenotypically distinct subpopulations distinguished by their opaque or translucent colony phenotypes under oblique lighting. The rate of interconversion between opaque and translucent variants is activated in a density-dependent manner and can approach 30-40%. Multiple phenotypic differences exist between opaque and translucent variants, including biofilm formation, motility, quorum-sensing signal production and capsule thickness. We demonstrate that the opaque variant is highly virulent in a mouse lung model of infection. In contrast, the translucent variant is rapidly killed *in-vivo* and highly susceptible to host defenses such as cationic antimicrobial peptides (CRAMP, LL-37), lysozyme and oxidative stress. RNA-Seq analysis has demonstrated that 114 genes were differentially regulated between opaque and translucent variants. The most highly upregulated gene in the translucent variant was a TetR-type transcriptional regulator ABUW_1645. Constitutive expression of this regulator in the opaque variant converted cells to the translucent state, locked them in this state and abrogated virulence. Therefore, this regulator serves as a central switch to drive the opaque variant to the translucent state. In human bloodstream infections, only the opaque variant was isolated further confirming the importance of this variant in virulence. A number of chromosomal and plasmid encoded loci have been identified that impact the rate of switching and the role of several of these genes in switching will be discussed.

**SS3-1**

**Mechanism of antibacterial toxin delivery by the bacterial type VI secretion system**

John WHITNEY, McMaster University, D. QUENTIN[2], S. AHMAD[1], S. RAUNSER[2], [1]McMaster University, [2]Max Planck Institute of Molecular Physiology

The type VI secretion system (T6SS) mediates antagonistic interactions between contacting bacterial cells. This molecular machine secretes antibacterial effector proteins by undergoing cycles of extension and contraction; however, how effectors are loaded into the T6SS and subsequently delivered to target bacteria remain poorly understood. Here, using electron cryomicroscopy, we determined the structures of the membrane protein effector Tse6 loaded onto the T6SS spike protein VgrG1 in solution and embedded in lipid nanodiscs. Tse6 stability in the absence of membranes requires the chaperone EagT6, two dimers of which interact with the hydrophobic transmembrane domains of Tse6. EagT6 is not directly involved in Tse6 delivery but rather is crucial for its loading onto VgrG1. VgrG1-loaded Tse6 spontaneously enters membranes and its toxin domain translocates across a lipid bilayer indicating that effector delivery by the T6SS does not require puncturing of the target cell inner membrane by VgrG1.

**SS3-2**

**Branching out: new horizons in *Streptomyces* development**

Marie ELLIOT, McMaster University, SE JONES[1], C PHAM[1], M ZAMBRI[1], DL SEXTON[1], D CRISANTE[1], [1]McMaster University

*Streptomyces* are sporulating, antibiotic-producing soil bacteria that are renowned for both their metabolic and developmental complexity. Their classical life cycle is characterized by a series of distinct, morphological stages. Growth initiates with spore germination, after which vegetative hyphal filaments are formed through hyphal tip extension and branching. Aerial hyphae are then raised above the vegetative cells, and these aerial cells are transformed into chains of dormant spores. Notably, *Streptomyces* is literally rooted in place during its vegetative growth. Recently, we have discovered a novel mode of *Streptomyces* growth that sees them traverse solid surfaces, with cells that appear to be distinct from the traditional 'vegetative' or 'aerial' cell designations. This 'exploratory' growth occurs in response to distinct metabolic cues, including low glucose and high amino acid concentrations. Remarkably, we have determined that exploring cells produce a volatile metabolite that (i) changes the pH of its surrounding environment, (ii) promotes exploration of more distantly positioned streptomycetes, and (iii) impacts the growth of other soil-dwelling bacteria. We have identified this metabolite as trimethylamine (TMA), and have shown that TMA alone can promote *Streptomyces* exploration. We are working to understand the exploration process from genetic, biochemical and ecological perspectives.

**SS3-3**

**Transient osmotic perturbation causes long-term alteration to the gut microbiota**

Carolina TROPINI, Stanford University School of Medicine, J.L. SONNENBURG[1], [1]Stanford University School of Medicine

Physical perturbations are prevalent in the bacterial world. Changes in the environmental mechanical properties, temperature, pH, or osmotic pressure apply broad spectrum stresses to bacterial communities and drive evolution. Specifically, in the human gut, osmotic stress is a common disturbance caused by food intolerance, malabsorption, and widespread laxative use. We assessed the resilience of the gut ecosystem to osmotic perturbation at multiple length and time scales. Osmotic stress caused consistent, lasting changes to human and mouse microbiotas in a mouse model, leading to the extinction of highly abundant taxa and expansion of less prevalent members. Using quantitative imaging, we showed that the mucosal interface separating bacteria from the epithelium was decimated during osmotic perturbation, but rapidly recovered when osmotic stress was removed. The immune system was also impacted, with temporary changes in cytokine levels and a lasting IgG response against commensal bacteria. Increased osmolality prevented bacterial growth in vitro, suggesting a host-independent mechanism for observed extinction events. Environmental availability of microbiota members that would otherwise go extinct restored composition to the pre-treatment state. These findings demonstrate that even mild osmotic perturbance can cause lasting changes to the microbiota and host, and lay the foundation for developing interventions that can increase system-wide resilience.

## SESSION IV: Structural Biology and Chemogenomics
## Tuesday, June 19th, 2018
## 10:45 AM – 12:15 PM

**IS 05**

**Bacterial pathogens rewriting ubiquitination pathways in the host**

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Ubiquitination is conserved eukaryote-specific signalling cascade culminating in the attachment of one or multiple copies of a small protein called ubiquitin to a target protein. Depending on the nature of ubiquitin modification arrangement it triggers either proteasomal degradation or a change in activity of target protein serving as the major signal transduction mechanism. Though bacteria lack ubiquitination, many bacterial pathogens have evolved specific pathogenic factors, called "effectors", that are secreted inside the host cell to effectively manipulate this process. Here we present our discovery and molecular characterisation of ubiquitination-manipulating effectors in the arsenal of pathogenic *Escherichia coli* and *Legionella pneumophila*. Combined, our data reveals novel molecular mechanisms in pathogenic bacteria's arsenal of host manipulation and highlights the complex regulatory mechanisms integral to bacteria's pathogenic strategy.

**SS4-1**

**Sporulation in Gram-negative bacteria**

Elitza TOCHEVA, Université de Montréal

Sporulation is a complex morphological process induced during starvation and it invlves extensive membrane remodeling. The process begins with DNA replication, chromosome segregation and packing, asymmetric positioning of the Z-ring, and septation. This yields a mother cell and a daughter cell, or "prespore", that are separated by a double-membraned septum. After septum formation, the mother cell engulfs the prespore in a process morphologically similar to phagocytosis. Finally, when the mother cell lyses, a mature spore is released. When favorable conditions return, the spores germinate and new progeny emerge via outgrowth. For decades, the model organism for studying both sporulation and the "Gram-positive" cell type architecture has been the bacterium *Bacillus subtilis* [1]. It is a member of the phylum Firmicutes and is surrounded by a single membrane and a thick layer of peptidoglycan. *Acetonema longum* is also a member the Firmicutes and forms endospores, however, the bacteria are surprisingly "Gram-negative": their cell envelope is composed of a thin layer of peptidoglycan enveloped by two membranes [2] We used cryo electron tomography (cryo-ET) to characterize sporulation in *A. longum* and compare it to *B. subtilis*. Cryo-ET provides 3-dimensional volumes of biological samples at macromolecular resolution [3-4]. Images of vegetative, sporulating and germinating cells revealed that both *B. subtilis* and *A. longum* bacteria produced spores that were surrounded by two membranes. Furthermore, in both cases the two membranes originated from the inner/cytoplasmic membrane of the mother cell. Some time between mid to late spore development and germination, *B. subtilis* loses its outer spore membrane to become a monoderm, "Gram-positive" vegetative cell, whereas *A. longum* retains both spore membranes and the outer spore membrane emerges as an OM. Therefore, endospore formation offers a novel hypothesis for how the bacterial OM could have evolved: a primordial monoderm cell may have first developed the ability to form endospores, and then this process could have given rise to diderm vegetative cells[3]. Our hypothesis that a diderm sporulating cell could have been the last common ancestor of all bacteria could explain the cell envelope distribution and diversity in modern bacterial phyla by subsequent loss-of-function (OM or ability to sporulate) events.

**References:**

[1] Tocheva, E. I., et al (2013). Peptidoglycan transformations during Bacillus subtilis sporulation. Mol Microbiol, 88(4), 673-686.
[2] Tocheva, E. I., et al (2011). Peptidoglycan remodeling and conversion of an inner membrane into an outer membrane during sporulation. Cell, 146(5), 799-812.
[3] Tocheva, E. I., Ortega, D. R., & Jensen, G. J. (2016). Sporulation, bacterial cell envelopes and the origin of life. Nat Rev Micro, 14, 535-542.

**MGC 003**

**Structural Characterization of HP0175 from *Helicobacter pylori* Reveals an Extension of Chaperone Helices Upon Target Interaction**

Gerald AUDETTE, York University, A YASEEN[1], [1]York University

Within a host, pathogenic bacteria employ several mechanisms that enhance their survival and motility. *Helicobacter pylori* secretes several virulence factors, including VacA, CagA, -glutamyltrasnpeptidase and HP0175.The secreted HP0175 has been shown to induce cell apoptosis through a cascade of mechanisms initiated through binding to Toll Like Receptor 4 (TLR4). HP0175 is classified as a parvulin-like peptidyl-prolyl *cis,trans*-isomerase (PPIase) involved in the isomerization of proline-containing peptide bonds. We have crystallized apo-HP0175 and determined the structure to 2.09 Å resolution. A comparison to the indole-2-carboxylic acid bound HP0175 form of the protein reveals that the N- and C-terminal helices of the chaperone domain extend upon engagement of the catalytic residues in the binding pocket by the inhibitor. Further, chromatographic and mass spectrometric analysis of HP0175 reveals that dimerization of the active form of the protein is dependent on ionic strength. These structural observations support the finding in other parvulins that the N- and C-terminal helices stabilize proteins undergoing catalysis by the PPIase domain, as well as suggest a means by which HP0175 could bind to TLR4, thereby initiating an apoptotic cascade in infected cells.

**SS4-3**

**The conserved prophage protein Paratox blocks activation of the quorum sensing transcriptional regulator ComR in *Streptococcus***

L MASHBURN-WARREN, The Research Institute at Nationwide Childrens Hospital, SD GOODMAN[1], MJ FEDERLE[2], Gerd PREHNA[3], [1]The Research Institute at Nationwide Childrens Hospital, [2]University of Illinois at Chicago, [3]University of Manitoba

Horizontal gene transfer is an important means of bacterial evolution. This includes natural genetic transformation, where bacterial cells become “competent” and DNA is acquired from the extracellular environment. Natural competence in many species of *Streptococcus* is regulated by quorum sensing via the ComRS receptor-signal pair. ComR is an Rgg-like transcriptional regulator that is activated upon binding the mature form of the ComS peptide pheromone, or XIP. The ComR-XIP complex induces expression of the alternative sigma factor SigX, which targets RNA polymerase to CIN-box promoters (TACGAATA) for the activation of genes involved in DNA uptake and recombination. In addition to genes of the competence regulon, the prophage gene paratox (*prx*) also contains a CIN-box, and here we demonstrate it to be transcriptionally activated by XIP. *prx* genes are widely distributed in *Streptococcus* at terminal ends of prophage and are adjacent to a toxin. These toxins include superantigens (SpeA), which are involved in GAS invasive diseases such as necrotizing fasciitis and streptococcal toxic shock syndrome. Additionally, *prx* is at a recombination hot-spot for the prophage lysogenic conversion module such that *prx* and the adjacent toxin are exchanged between phages as one genetic cassette. Interestingly, we found that a deletion of *prx* in *Streptococcus* (MGAS5005) causes a 100,000-fold increase in transformation efficiency by electroporation, but not natural transformation, which remains elusive for cultured *Streptococcus pyogenes*. *In vitro* experiments demonstrate that Prx binds ComR directly, blocks the binding of XIP to ComR, and prevents ComR from interacting with DNA. Additionally, an X-ray crystal structure of Prx reveals a unique fold with low homology to known proteins, which implies a novel mechanism of action for the binding and inhibition of ComR. Overall, our results suggest Prx functions to inhibit the acquisition of new DNA by *Streptococcus*, showing a previously unknown and interesting intersection between horizontal gene transfer by transduction and by natural competence in *Streptococcus.*

## SESSION V: Microbiome
## Tuesday, June 19th, 2018
## 10:45 AM – 12:15 PM

**IS06**

**Using comparative genomics to identify the genetic basis of commensal effects on plant health**

Cara HANEY, University of British Columbia

Plant root-associated microbial communities ("rhizosphere microbiome") influence plant growth and defense. Closely-related bacteria can have dramatically different effects on plant growth and range from pathogenic to mutualistic. As a result, function of a community cannot be predicted by taxonomic (e.g. 16S rRNA sequencing) methods alone. Using *Pseudomonas fluorescens*and *Arabidopsis* as a tractable rhizosphere microbiome model, we are using a combination of comparative genomics and functional assays to correlate microbiome function with the presence of certain genes in the plant microbiome. Using these approaches, we have identified the genetic basis of 1) *Pseudomonas* modulation of plant immunity and 2) that *P. fluorescens* can transition from an opportunistic pathogen through gain and loss of genomic islands via homologous recombination. We have identified closely-related strains (>97% identical by 16S rRNA) of *P. fluorescens* that induce systemic resistance (ISR) or susceptibility (ISS) to foliar pathogens. Using a combination of comparative genomics and functional assays we have identified a bacterial spermidine synthase (*speE)* gene that is uniquely present in ISS strains. We have deleted the *speE* gene and found that it is necessary for the ISS phenotype, and purified spermidine can phenocopy ISS strains. This indicates that single bacterial genes can underlie effects on host immunity. In addition to bacterial genes involved in modulation of systemic defense, we have identified *P. fluorescens* genes that are involved in transition from pathogenic to commensal lifestyles within *P. fluorescens*. Using the same comparative genomics platform, we have found that this transition is mediated by homologous recombination leading to gain and loss of horizontally transferred genomic elements. This work suggests that homologous recombination may be an evolutionary mechanism driving lifestyle changes in closely-related bacteria. Collectively, this work will inform our understanding of bacterial transitions from free living to host-associated, and how the plant microbiome affects plant health.

**SS5-1**

**Unmasking the contribution of uncultivated species and biovars in the vaginal microbiome to preterm birth**

Laura SYCURO, University of Calgary, A WISER[2], I LIACHKO[3], TP JAYAPRAKASH[1], H KHAN[1], S THAKUR[1], JN BURTON[4], H RAMAY[1], JL GOLOB[2], J SHENDURE[4], MJ DUNHAM[4], DN FREDRICKS[2], [1]University of Calgary, [2]Fred Hutch Cancer Research Center, [3]Phase Genomics, [4]University of Washington

The human vaginal microbiome contains many uncultivated bacterial species that are prevalent in women with bacterial vaginosis (BV) and implicated in preterm birth. From a single BV(+) vaginal metagenome with a high density of uncultivated species (12 of 30 spp. >0.1% relative abundance), we segregated 28 draft genome sequences using intracellular chromosome linkage analysis (Hi-C metagenomics). Genome quality approached that of isolate genome sequences (median completeness and contamination: 93% and 3%), with highly contiguous genome sequences (n50: 30-300 kb) representing a novel phylum, a novel class, and two novel families/genera. Among these are the first genome sequences for the uncultivated Clostridiales species BVAB1 and BVAB2, which are highly prevalent in BV(+) women. Both have been epidemiologically tracked for 13 years and repeatedly associated with preterm birth, a linkage strengthened by our discovery of a distinctive array of virulence genes in each species. BVAB1 encodes a full flagellar apparatus with 5 divergent copies of *flaA*, suggesting it is may employ isogenic switching to evade the immune system. BVAB1 also encodes chemotaxis machinery and a putative type IV secretion system. In contrast, BVAB2 encodes over 30 orthologues of virulence factors in *Listeria monocytogenes*, most of which are involved in its intracellular lifestyle. These include 7 putative internalin proteins known to be critical for *L. monocytogenes* to invade cells and cross the placental barrier. These findings suggest BVAB1 and BVAB2 employ known mechanisms of ascension and invasion to overcome the anatomical and immunological barriers safeguarding the intrauterine cavity. Hi-C also resolved 10 strains of *Atopobium vaginae* and *Gardnerella vaginalis*, which are the primary biofilm-forming species in the vagina and associated, in high abundance, with preterm birth. Since *G. vaginalis* was first cultivated in the mid-1950's, >100 bacterial isolates have been genotyped (half with WGS) as belonging to one of four subspecies with

differing virulence potential. It was thus surprising to discover that our sample's dominant strains belonged to two novel subspecies, together increasing the number of genes in the species pangenome by ~30%. We demonstrated that *A. vaginae* also exhibits extreme strain heterogeneity, with two *A. vaginae* strains representing the two known subspecies and a third strain representing a novel subspecies that expanded the species pangenome by ~50%. This work demonstrates that high quality reference genomes generated using Hi-C can be instrumental for detecting uncultivated biovars, even within well-characterized species, and launching studies aimed at understanding the microbiome's contribution to human pathologies such as intraamniotic infection and preterm birth.

**SS5-2**

**Microbial fibre breakdown and cross-feeding in the infant gut**

Jennifer STEARNS, McMaster University, C LONG[1], A PATEL[1], [1]McMaster University

**Introduction:** The infant gut is rapidly colonized by microorganisms soon after birth, and the composition of the microbiota is dynamic in the first year of life. Although a stable microbiome may not be established until 1 to 3 years after birth, the infant gut microbiota appears to be an important predictor of health outcomes in later life. The benefits of breastfeeding are well known, as are the benefits of good nutrition throughout childhood, yet less is known about how the diet in the first year of life is impacting the gut microbiome. Dietary fibre is likely impacting the microbial ecology of the infant gut in a number of ways that have yet to be explored. We are studying how fibre degrading microbes might be acting as keystone species and engaging in cross-feeding interactions that affect overall community functioning in this dynamic environment. We also hope to study how these important gut colonizers are driving succession of the microbial communities. Here we present early data on the diversity in types of fibre breakdown capacity of bacterial isolates from the infant gut. We used both molecular and culture based methods to study these ecologically relevant traits within the context of the infant gut environment. **Methods and Results:** We used 16S rRNA gene and shotgun metagenomic sequencing to profile the microbial community within 1 year old infant stool and get a list of potential genes for fibre breakdown. We then used targeted and enrichment culture to collect an average of 200 fibre-degrading microbial isolates per sample. We developed simple differential agar assays to measure the amount of breakdown of five fibre substrates (starch, pectin, inulin, cellulose and caboxymethyl cellulose). We next profiled the ability of each isolate to grow on the fibre substrates as the sole source of carbon and the metabolites produced from fibre fermentation. We targeted secondary fermenters from the same samples to measure cross-feeding interactions. **Conclusions:** By integrating functional assays with culture and molecular methods we were able to make observations that were not possible at the 16S rRNA gene level alone and were incomplete at the gene level. We demonstrate the deep diversity in fibre metabolism within the 1-year-old infant gut microbiome and identify potential cross-feeding relationships that likely have important impacts on the microbial community as a whole. The gut microbiome is the interface between the diet and the host. This work is uncovering the role of diet on shaping microbial communities and the role of communities on metabolizing the diet, during infancy, which is an important developmental time.

**SS5-3**

**Experimental Microbial Dysbiosis Does Not Accelerate Disease Progression in SIV Infection**

Jason BRENCHLEY, National Institutes of Health, USA

HIV-1 infection is associated with inflammation that is not fully reversed with antiretroviral therapy. Inflammation in HIV-infected individuals is associated with enrichment of disease-associated intestinal microflora, namely, Proteobacteria. Although several studies have indicated that therapeutic administration of probiotic species has immunological benefit, an empirical assessment of the contribution of microbial dysbiosis to disease progression is lacking. To assess the contribution of bacterial GI tract dysbiosis to lentiviral disease progression, we administered the antibiotic vancomycin to macaques pre- and post-SIV-infection. Vancomycin treatment resulted in a significant and progressive increase in the relative frequency of fecal Proteobacteria and decrease of Firmicutes during chronic SIV infection. Surprisingly, no adverse differences in viremia, immune activation, nor other canonical indicators of disease progression were observed in experimental animals throughout SIV-infection. Our results demonstrate that key features of HIV-associated inestinal dysbiosis are ancillary to disease progression in a non-human primate model of HIV-1 disease progression.

## SESSION VI: Biotechnology for Biofuels and Bioproducts
## Tuesday, June 19th, 2018
## 10:45 AM – 12:15 PM

**IS 07**
**Microbial Cellulose Utilization: From Applications to Fundamentals and Back Again**
LEE LYND, Thayer School of Engineering, Dartmouth, USA

Microbially-mediated transformations of lignocellulose play a key role in natural ecosystems, managed systems such as the rumen, anaerobic digestion, and ensiling, and could also be the basis for low-carbon fuel production in the future. Departing from a desire to gracefully incorporate transport fuel production into the carbon cycle, exploration into fundamental aspects of microbial cellulose utilization will be described. These aspects include bioenergetics, as well as documenting and understanding cellulosic biomass deconstruction by various biological systems (e.g. enzymes, pure and mixed cultures) under various conditions. Applied topics will also be addressed, including development and use of genetic systems, and strategies to augment biological conversion of lignocellulose. Perspectives will be offered on general issues such as the interplay between applied and fundamental research, how seemingly small differences in the questions posed can lead to substantial differences in the answers obtained, and opportunities and challenges related to biotechnology using non-model microbes.

**SS6-1**
**Metagenomics of Microbial Dechlorinating Consortia**
Elizabeth EDWARDS, University of Toronto, O MOLENDA[1], L PUENTES-JACOME[1], W QIAO[1], F LUO[1], P-H WANG[1], L LOMHEIM[1], S DWORATZEK[2], K MAXWELL[1], N MORSON[1], [1]University of Toronto, [2]SiREM

Groundwater contamination is a serious threat to global health and prosperity. Petroleum hydrocarbons, industrial solvents, pesticides, herbicides and metals are some of the most frequent culprits. Some microbes have evolved and adapted to transform or detoxify contaminants in the environment. A fascinating group of subsurface microorganisms, collectively referred to as organohalide-respiring bacteria, are significant players in the global halogen cycle. Certain species, such as *Dehalococcoides*, can dechlorinate the major dry-cleaning solvent tetrachloroethene and the common industrial solvent trichloroethene to the benign product ethene. Remarkably, these organisms obtain energy for growth from dechlorination and several successful demonstrations of bioaugmentation, where an aquifer is inoculated with culture, have lead to the development of a commercial market for such dechlorinating cultures. There are many halogenated organic molecules that are substrates for microbial reductive dehalogenation. Through enrichment cultures and metagenome investigations, we are learning more about interspecies interactions in these remarkable, ubiquitous, microbial communities, and their specialized enzymes that catalyze reductive dehalogenation reactions. Examples of such microbial communities that perform valuable ecosystem services will be highlighted.

**AEM 017**
**Polyhydroxyalkanoate (PHA) production by *Pseudomonas chlororaphis* PA23 is controlled by Quorum Sensing and the Global Regulator ANR**
Teresa DE KIEVIT, University of Manitoba, P SHARMA[1], D LEVIN[1], [1]University of Manitoba

*Pseudomonas chlororaphis* strain PA23 is a biocontrol agent capable of suppressing disease caused by the fungal pathogen *Sclerotinia sclerotiorum.* A number of compounds contribute to fungal antagonism, including antibiotics and degradative enzymes. PA23 is also capable of synthesizing polyhydroxyalkanoate (PHA) polymers as carbon and energy storage compounds under nutrient-limiting conditions. In PA23, expression of biocontrol metabolites is controlled by a complex regulatory cascade that functions at both the transcriptional and posttranscriptional level. We have previously reported that the PhzRI quorum-sensing (QS) system and a global regulator ANR (anaerobic regulator) are both essential for antibiotic and degradative enzyme production by this bacterium. Like other pseudomonads, the genes responsible for PA23 PHA production are arranged as a cluster known as the *pha* operon. At present, regulators of the PA23 *pha* genes have yet to be identified. The objective of this study, therefore, was to determine whether QS

and/or ANR play a role in PHA expression. For this analysis, two QS-deficient strains (PA23*phzR*and PA23AHL) and an *anr* mutant were investigated. We discovered that when cultured in Ramsay's Minimal Medium with glucose or octanoic acid as the carbon source, all three strains exhibited reduced PHA accumulation compared to the wild type. Moreover differences in the PHA monomer composition for the QS-deficient and *anr* mutant was observed on both carbon sources. Because QS and ANR function at the level of transcription, we monitored *pha* gene expression using quantitative real time PCR. RNA extracted from the wild-type and three derivative stains enabled us to analyze transcription of *phaC1, phaZ, phaC2, phaD, phaF,* and *phaI*. With the exception of *phaC2*, which encodes one of the two PHA synthases, all of the other genes were downregulated in the mutant backgrounds, consistent with reduced PHA accumulation. Collectively these findings indicate that in addition to controlling key antifungal metabolites, the PhzQS system and ANR govern expression of PHA production in biocontrol strain PA23.

**SS6-3**

**Dual culture of bacterium and fungus to increase the recovery of nutrients from agriculture byproducts**

JRA KUTTIYATVEETIL, University of Saskatchewan, P MITRA[1], MT NICKERSON[1], Takuji TANAKA[1], [1]University of Saskatchewan

We have shown that combinations of solid-state fermentation (SSF) and black soldier fly larvae (BSFL) culture is a strong tool to recover unutilized nutritional substance from agri-byproducts. In order to further improve their recovery using this strategy, we employed dual strain SSF based on the results from single strain SSF. **Methods:** Borage and flaxseed meal cakes were milled and adjusted to a 75% moisture level. After sterilized with an autoclave, the meal cakes were inoculated with *Lactobacillus plantarum*, *Aspergillus niger* and *A. oryzae*. Dual strain fermentation (DuF) with two combinations of these strains (*L. plantarum + A. niger* (Lp+An) and *L. plantarum + A. oryzae* (Lp+Ao)) were conducted at 37 °C for 4 days with respective single strain fermentation (SiF) for comparisons. The proximate analysis was conducted in order to determine the levels of protein, lipids, ash and carbohydrate after fermentation. The fermented meals were the fed to BSFL for 12 days. During the BSFL culture, the larvae and spent feeds were sampled at every 4 days. The growth of larvae, gain in the larval biomass, and consumption of nutrients in fermented meals were monitored with determination of proximity of nutrients and physical size measurements. **Results and Discussion:** SiF and DuF increased both protein and lipid contents in fermented borage meals from 26.7%/3.2% to 30.1-33.2%/3.8-6.8%; whereas changes in flaxseed meals are 33.1%/6.6% to 33.1-42.9%/2.7-4.2%. DuF meals allowed the larvae grew to 230-300 $mm^3$ from 26 $mm^3$ in 8 to 12 days of feeding, whereas unfermented feed have BSFL grew to 200-230 $mm^3$ in the same period. SiF supported the growth to 230-260 $mm^3$ in 12 days. The both combinations of DuF borage meals showed the best growth where 300 $mm^3$ was achieved in 8 days. During these growths, about 1/2 ~ 2/3 of proteins and virtually all lipids disappeared from spent feeds. This nutrients were gained by the BSFL: Protein contents per larva increase from 2.9 mg protein to 36.3 mg in Lp+Ao DuF borage meals and to 35.4 mg in Lp+Ao DuF flaxseed meals. Overall the gain in the protein and lipid levels significantly increased by the DuF fermentation compared to SiF meals. This gain is mostly because of the increase amount and quality of proteins and lipids through SSF, and in addition, we speculate the accessibility to proteins, lipids and carbohydrates are modified by the SSF. The results indicated that the combination of bacterium and fungus strains are effective to recover nutrients from agriculture byproducts.

## SESSION VII: Antimicrobial Resistance
## Wednesday, June 20th, 2018
## 8:30 - 10:00 AM

**IS 08**

**Chasing carbapenemase carrying plasmids through patients and plumbing**

Amy MATHERS, University of Virginia, A SHEPPARD[2], S KOTAY[1], K VEGESANA[1], [1]University of Virginia, [2]Oxford University

Antibiotic resistance is a major public health concern. In *Enterobacteriaceae*, resistance genes are often located on conjugative plasmids, which mediate horizontal resistance dissemination. There has been increasing recognition of the role of waste water in hospital premise plumbing acting as a reservoir for carbapenemease producing Enterobacteriaceae with potential for spread to patients. However, determining specific pathways of plasmid transmission within a clinical setting is challenging especially with an environmental reservoir and mobile genetic elements are involved. The *bla*$_{KPC}$ gene confers resistance to last-line carbapenems, and is usually found within the 10 kb Tn3-based transposon Tn4401. Applying novel microbiologic and bioinformatics techniques to understand the movement of a resistance gene through a hospital system between patients and the wastewater environment across multiple species and plasmids we begin to understand some transmission chains. Through a combination of short- and long-read whole genome sequencing techniques to investigate *bla*$_{KPC}$ plasmid structures demonstrates how these tools can be utilized for tracking a complex plasmid transmission across bacterial species. Using a multi-pronged approach of a ex situ model of transmission in an unique "sink lab" we have realized some of the transmission dynamics of bacteria from sink drains to the surrounding area. Lastly, we deploy environmental mitigation in the hospital with success and decrease patient acquisition of KPC producing bacteria by half.

**AEM 064**

**Extended spectrum β-lactamases producing bacteria isolated from feces of gilts and sows reared in conventional and antibiotic-free settings**

Dominic POULIN-LAPRADE, Sherbrooke Research and Development Centre of Agriculture and Agri-Food Canada, N. GAGNON[1], A. TURCOTTE[1], E. TOPP[2], J.J. MATTE[1], G. TALBOT[1], [1]Sherbrooke Research and Development Centre of Agriculture and Agri-Food Canada, [2]London Research and Development Centre of Agriculture and Agri-Food Canada

Antibiotic resistance is a recognized menace which will most likely surpass cancer as the leading cause of death by 2050. In Canada, approximately 80% of all the antibiotics sold are administered to livestock animals, promoting the development and spread of antibiotic resistance genes in food-borne pathogens. Among them are the *Enterobacteriaceae* producing extended spectrum β-lactamases (ESBLs), which are secreted enzymes conferring resistance to most of the available antibiotics targeting the bacterial cell wall. The main objective of this study was to assess and characterize these antibiotic resistant bacteria in feces of gilts and sows reared in commercial facilities. At one time point, feces of three groups of animals were sampled: (i) 15 gilts in quarantine after their transport from the multiplication farm, (ii) 14 third-parity sows reared with conventional practices and (iii) 15 third-parity sows reared in antibiotic-free settings. The total and resistant bacteria were quantified by plating serial dilutions of feces onto MacConkey II medium (MAC) supplemented or not with 2 µg/ml cefotaxime (CTX), a third generation cephalosporin, or 8 µg/ml chlortetracycline (TET), the antibiotic most widely used in swine production in Canada. The phenotypic resistance profile of over 1,500 isolates was screened and the minimal inhibitory concentration of 18 antibiotics was determined for a subset of multidrug resistant organisms using the disk diffusion method following the Clinical & Laboratory Standards Institute guidelines. When compared to feces excreted by conventional sows, the gilts feces contained significantly lower counts of lactose-fermenters growing on MAC supplemented or not with TET, but they carried a higher percentage of bacteria resistant to TET and/or CTX. Surprisingly, feces from sows reared in antibiotic-free settings also showed a higher proportion of TET-resistant bacteria than in conventional settings. As for the CTX resistant colony counts, no difference was observed between the three groups of animals. Organisms producing ESBLs were isolated from most of the feces samples. All the animals received a dose of ceftiofur at 3 days of age and cocktails of antibiotics as additives to their rations in nursery. Exposure to these antibiotics may exacerbate the development of

resistant bacteria, explaining why the gilts carry proportionally more resistant bacteria in their gut than the older sows, for which the lack of exposure over a longer period may have allowed the antibiotic-susceptible populations to thrive. Nevertheless, ESBLs-producing *Enterobacteriaceae* were isolated from the three groups of animals. As contamination of carcasses with *Enterobacteriaceae* is very common, there is a potential for these resistant bacteria to make their way towards fresh meat products. This is especially worrying as ESBLs provide resistance against antibiotics of very high importance in human medicine with limited availability of alternative treatments.

**SS7-2**

**What determines the rate of antibiotic resistance evolution?**

NG GHADDAR, Concordia University, MH HASHEMIDAHAJ[1], Brandon FINDLAY[1], [1]Concordia University

The steady rise of antibiotic-resistant bacteria is a global threat, rendering valuable antibiotics obsolete and increasing morbidity and mortality. These bacteria rapidly emerge following introduction of each new antibiotic in the clinic, and can in many cases naïve bacteria can be readily made resistant through *in vitro* studies. Due in part to these lab studies we now know many of the mutational pathways that can lead to antibiotic resistance, but many of the factors that control the rate of evolution remain unclear. Working with soft agar plates we have developed a compact evolution platform. Called soft agar gradient evolution (SAGE), this system was validated by generating mutants of *Escherichia coli* resistant to twelve antibiotics spanning each of the predominant classes with activity against Gram-negative bacteria. We then took advantage of the flexibility of this system to examine the kinetics of resistance evolution, determining why resistance to some antibiotics is generally difficult to evolve in some *in vitro* systems. This presentation will introduce these results and cover other recent discoveries with the SAGE system.

**II 051**

**The Comprehensive Antibiotic Resistance Database and the Resistance Gene Identifier – Prediction of Antimicrobial Resistance Genes for Genomic and Metagenomic Sequencing Data**

A.R. RAPHENYA, McMaster University, B. ALCOCK[1], K.K. TSANG[1], A.J. SHARMA[1], T.T.Y. LAU[1], A. HERNANDEZ-KOUTOUCHEVA[2], Andrew MCARTHUR[1], [1]McMaster University, [2]National Autonomous University of Mexico

The growing crisis of antimicrobial resistance (AMR) is driven by the evolutionary capacity of the microbial resistome and human-made selective pressure. It is further exacerbated by a weakened drug discovery pipeline. In the absence of a robust pipeline of new drugs coming to market, understanding the genomic basis of resistance and its movement through bacterial and patient communities is essential for judicious management of increasingly scarce antibiotics and to guide new drug discovery. However, phenotypic criteria dominate the epidemiology of antibiotic action and effectiveness. There is a poor understanding of which antibiotic resistance genes are in circulation, which a threat, and how clinicians and public health workers can manage the crisis of resistance. Gaps in our capacity to detect and respond to antimicrobial resistance threats, combined with lack of systematic national and international surveillance, can only be addressed by application of advanced technologies. As such, we have developed the Comprehensive Antibiotic Resistance Database (CARD), Antibiotic Resistance Ontology, and Resistance Gene Identifier (card.mcmaster.ca) as first generation tools for a world where DNA sequencing of AMR pathogens has become commonplace at every hospital, clinic, and outbreak. We have developed the Resistance Gene Identifier (RGI) software to predict total resistome based on bioinformatics models covering the diversity of resistance mechanisms, including acquired resistance genes, adaptive mutations in both protein-coding and ribosomal RNA genes, and up-regulation of efflux. The RGI analyzes sequences under three paradigms – Perfect, Strict, and Loose (a.k.a. Discovery). The Perfect algorithm is most often applied to clinical surveillance as it detects perfect matches to the curated reference sequences and mutations in the CARD. In contrast, the Strict algorithm detects previously unknown variants of known AMR genes, including secondary screen for key mutations, using detection models with hand curated similarity cut-offs to ensure the detected variant is likely a functional AMR gene. The Loose algorithm works outside of the detection model cut-offs to provide detection of new, emergent threats and more distant homologs of AMR genes, but will also catalog homologous sequences and spurious partial hits that may not have a role in AMR. Combined with phenotypic screening, the Loose algorithm allows researchers to hone in on new AMR genes. Lastly, the RGI can predict AMR determinants from finished genome sequences, high-quality genome assemblies, low quality/coverage assemblies,

metagenomic merged reads, small plasmids or assembly contigs, and new tools are in development for annotation of metagenomics reads with associated prediction of pathogen-of-origin. The RGI is available both as an online tool, with data visualizations, or a downloadable command-line tool for bulk analyses.

## SESSION VIII: Unusual Microbes and Extreme Environments
## Wednesday, June 20th, 2018
## 8:30 - 10:00 AM

**IS 09**

**Unusual microbes in relatively normal environments: bringing the extremophiles home**

Jennifer BIDDLE, University of Delaware

Once upon a time, archaea were considered extremophiles, until time after time they were sampled from relatively normal environments. Now, we know that archaea are relatively ubiquitous, even growing in a human belly button after the "relatively" normal condition of not showering for months. What other "extremophiles" are lurking nearby? Organisms such as the archaea and large sulfur oxidizers were once considered the inhabitants of the deep sea. Yet, suggestions of a worldwide distribution mean that they must be found in many areas. In order to investigate this, we sampled the habitats in our backyard. Through amplicon and metagenomic sampling, we see relatives of deep sea archaea bloom on large particles in a coastal river. Next, a bone placed in this flowing water grew a sulfur-oxidizing biofilm, which contains members of the Beggiatoa, sulfur-oxidizing Gammaproteobacteria, and eventually developed Epsilonproteobacteria. Surprisingly over the time course of this incubation, there is a reliable shift in the sulfur-oxidizer community, and even a bloom of rare Asgard archaea. Through a metagenomic-enabled perspective, we were able to assemble mulitple genomes of Beggiatoa, and compare them to other deep-sea strains and cultured laboratory freshwater strains. The genome comparisons suggest that there is an open genome of Beggiatoa globally, and many more genomes will be needed to fully understand the capability of that group. Within the Beggiatoa of the high salinity river water, we see that there are drastically different abundances in genes for regulation and community coordination, which lends some suggestions to why these organisms are so difficult to cultivate. With the backyard approach to understanding extremophiles, we can better prepare ourselves to understand unique strains in other environments, and potentially bring more organisms into cultivation in the laboratory.

**SS8-1**

**Bioconversion of Selenium and Tellurium oxyanions into nanomaterials by bacteria**

Raymond TURNER, University of Calgary, E PIACENZA[1], A PRESENTATO[1], S LAMPIS[2], G VALLINI[2], M CAPPELLETTI[3], R BORGHESE[3], D ZANNONI[3], [1]University of Calgary, [2]University of Verona, Italy, [3]University of Bologna, Italy

The oxyanion forms, particularly the oxidation IV state of Selenium (Selenite, $SeO_3^{2-}$) and Tellurium (Tellurite, $TeO_3^{2-}$) are bioavailable and toxic to most bacterial species. However, over the past decade, several species have been isolated that are able to tolerate these oxyanions and convert them to the less toxic and less bioavailable elemental forms of $Se^0$and $Te^0$that precipitate out. Recently it has been recognized that the precipitates are on the nanoscale. Thus the field has moved to see such strains as able to bioconvert these toxic metalloids into nanomaterials, thus simultaneously having the potential for bioremediation of chalcogen contaminated sites as well as being an eco-friendly alternative for the synthesis of Se and Te nanomaterials. We have explored a number of different bacterial strains to date including: *Escherichia coli*, *Bacillus mycoides* SelTE01, *Stenotrophomonas maltophilia* SelTE02,*Rhodococcus aetherivorans*BCP1, and *Rhodobacter capsulatus*. The nanomaterials that these strains produce are nanoparticles and nanorods of various sizes, dimensions, and crystalline structures. The physicochemical characteristics of the nanomaterial forms are dependent on a combination of the strain and different culturing conditions. In some cases, the nanomaterials are either secreted or produced extracellularly, for others the nanomaterials are found intracellularly. This process is of interest for an approach of bioremediation of Se/Te contaminated environments

through biosorption and conversion to a value-added product. Our work demonstrates the promise of using bacteria as green chemistry bionanofactories to produced various metal(lloid) nanomaterials.

**SS8-2**

**From acid mine drainage to enhanced bioleaching: understanding and applying microbial communities from extreme mine waste environments**

Nadia MYKYTCZUK, Laurentian University, E PRINCIPE[1], M KHAN[1], [1]Laurentian University

Sulfidic mine tailings harbour a diversity of microorganisms within both the oxidized and reduced zones. Undeterred by the conditions of low pH and high metal content, these organisms interact with minerals on a microscopic level and catalyze the oxidation/reduction of parent materials, the formation of secondary minerals, and subsequently affect the transformation of metals. In some instances we can promote these microbially driven reactions to help liberate or stabilize target minerals and elements but understanding the role of specific organisms is challenging without high resolution data. We demonstrate how genomics tools are being applied to 1) characterize the microbial communities present in mine waste environments and 2) how we can apply these to bioleaching applications for metal recovery. Unraveling the microbial activities in these extreme environments aims to find better ways of using microbial data and applying microbial consortia to managing and capitalizing on mine wastes. We will provide an overview of field to pilot scale data from projects with Vale, and BacTech Environmental with method development from MetagenomBio Inc. Clearly identifying the functional pathways that are active during mine waste weathering and leaching tests allows us to determine rate limiting steps that might be optimized with modifying operational parameters of existing and developing technologies. As the integration of established bioremediation and bioleaching technology and genomics mature with projects like these, it is anticipated that genomics tools and custom consortia will help refine bioleaching and allow for further innovation and application of this technology to a variety of mine wastes for effective metal recovery and remediation.

**SS8-3**

**Mutualistic cross-feeding during nutrient limitation in a synthetic bacterial coculture**

James MCKINLAY, Indiana University, AL MCCULLY[1], B LASARRE[1], [1]Indiana University

Microbial mutualisms based on cross-feeding of metabolites are widespread yet invariably face periods of nutrient limitation. How nutrient limitation impacts mutualist growth, cross-feeding levels, and mutualism dynamics remains poorly understood. We examined the effects of nutrient limitation within a mutualism using theoretical and experimental approaches with a synthetic anaerobic coculture pairing fermentative *Escherichia coli* and phototrophic *Rhodopseudomonas palustris*. In this coculture, *E. coli* and *R. palustris* resemble an anaerobic food web by cross-feeding essential carbon (organic acids) and nitrogen (ammonium), respectively. Organic acid cross-feeding stemming from *E. coli* fermentation of glucose continues in a growth-independent manner during nutrient limitation and provides essential maintenance energy for *E. coli*. Ammonium cross-feeding by *R. palustris* stemming from $N_2$ fixation is growth-dependent. When ammonium cross-feeding was limited by decreasing the $N_2$ supplied from 100% to as low as 6%, coculture trends changed yet coexistence persisted under both homogenous and heterogenous conditions. Even when *E. coli* growth was completely prevented by using a histidine auxotroph, growth-independent fermentation sustained the growth of *R. palustris*.Theoretical modeling indicated that growth-independent fermentation was crucial to sustain cooperative growth under conditions of low nutrient exchange. Thus we propose that growth-independent maintenance metabolism is an important mechanism by which mutualistic cross-feeding can establish and persist in the face of nutrient limitation.

## SESSION IX: Host – Microbe Interactions
## Wednesday, June 20th, 2018
## 8:30 - 10:00 AM

**IS 10**
**To Give or to Take: Bacterial Regulation of Conflicting Symbiotic Behaviors with Invertebrates**
Heidi GOODRICH-BLAIR, University of Tennessee-Knoxville

Entomopathogenic Steinernema nematodes employ Xenorhabdus bacteria symbionts to help kill insects and to support reproduction within the cadaver. Infective nematodes release their bacterial symbiont into the insect hemocoel, where the two allies face and overcome insect immunity. The bacterial symbiont then provides activities that support nematode reproduction through bioconversion of the insect cadaver. Nutrient depletion triggers development of the nematode into its colonized infective form to begin the cycle anew. Virulence and immune suppression have been investigated using X. nematophila bacteria, the symbiont of S. carpocapsae nematodes, infecting lepidopteran insects (e.g. Manduca sexta). The mutualistic and pathogenic behaviors ofX. nematophila symbionts are regulated by the transcription factor Lrp, which regulates the expression of many genes. I will discuss how this regulation helps coordinate expression of symbiotic traits during the Xenorhabdus-Steinernema life cycle.

**II 012**
**Adaptation of *Burkholderia cenocepacia* to the cystic fibrosis lung environment**
Silvia CARDONA, University of Manitoba, B KUMAR [1], TJ LIGHTLY[1], [1]University of Manitoba

Species of the *Burkholderia cepacia* complex (Bcc) colonize the lungs of people with cystic fibrosis (CF), producing a spectrum of clinical manifestations, ranging from asymptomatic carriage to the lethal "cepacia syndrome". Despite intense clinical research, the reasons why some CF patients succumb to Bcc infections while others do not are unknown. Major carbon and nitrogen sources supporting the CF microbiota are the amino acids present in the CF mucus. Our working hypothesis is that Bcc senses changes in amino acid availability eliciting different pathogenic responses. Using a clinically relevant Bcc species, *B. cenocepacia*, I will describe three overlapping virulence mechanisms triggered by amino acids: i) The number of flagella increases in media containing all amino acids; ii) Swimming motility and protease activity is upregulated in response to arginine and glutamate by CpdA-mediated decrease of c-di-GMP signaling; iii) Pathogenicity becomes dependent or independent of cepIR quorum sensing regulation when phenylalanine is metabolized through a phenylacetic acid degradation pathway that is interrupted at different steps. Intriguingly, recently published RNA-seq analysis of *B. cenocepacia* with high intracellular c-di GMP shows that c-di-GMP downregulates phenylacetic acid metabolism, suggesting that phenylacetic acid intermediates could be modulators of c-di-GMP signalling. The intertwining of mechanisms with different effects on pathogenicity in response to amino acid content suggests that complex, niche-specific, nutritional differences may explain the highly variable clinical outcomes observed in B. cepacia complex infections.

**II 011**
***Shigella* Type Three Secretion System reloaded**
N SILUÉ, University of Ottawa, A ALZAHRANI[1], W BAJUNAID[1], L PINAUD[2], François-Xavier CAMPBELL-VALOIS[1], [1]University of Ottawa, [2]Institut Pasteur

*Shigella flexneri* is a well-studied Gram-negative enteropathogen, which has been up to this day one of the gold standard model to comprehend the functioning of the Type Three Secretion System (T3SS) that it shares with many other bacteria. Despite decades of research many fundamental questions about the functioning of the T3SS and the role of secreted protein-effectors during host colonization remain unanswered. This has motivated both my individual effort as a postdoctoral fellow, and the collective efforts of my research group in the last couple of years. In the frame of the CSM/SCM annual meeting/conférence annuelle, I will show how we have used mass spectrometry and RNAseq to extend the panel of T3SS substrates in *S. flexneri*. These studies have allowed the identification of seven novel secreted proteins. Two of these are encoded on the chromosome and seem to belong to novel classes of effector. I will also

present how we have used a fluorescent Transcription-based Secretion Activity Reporter (TSAR) to delineate the activity of the T3SS inside infected host cells. The TSAR demonstrated that the T3SS oscillate between an on- and an off-state depending on the interaction of bacteria with plasma membrane-derived compartments. The prospect of using this type of reporter not only in human cell lines but also *in vivo* inside infected tissue will be briefly discussed. Furthermore, I will demonstrate how using the TSAR to re-explore old phenotypes has surprisingly led us to reconsider the cellular context and mechanisms leading to targeting of *Shigella* by autophagy, and particularly the role of the effector IcsB in this process. In brief, our findings have furthered the comprehension of *Shigella* pathogenesis and described a series of simple technical and conceptual tools to achieve this. I believe they should spark the interest of everyone curious about host-microbe interactions.

**SS4-3**

**A heme relay from human hemoglobin to SbnI, a regulator of staphyloferrin B biosynthesis in *S. aureus***

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The Isd cluster of *Staphylococcus aureus* encodes seven genes to enable growth on heme or Hb as a sole iron source. IsdB is a receptor on the bacterial surface that extracts heme from hemoglobin (Hb). From IsdB, heme is relayed into the cytosol where IsdG degrades the heme porphyrin ring to release iron. IsdB is important both for *in vitro* growth on Hb and in infection models and is also highly upregulated in blood, serum, and tissue infection models, indicating a key role of this receptor in bacterial virulence. SbnI is regulated the expression of the genes encoding for the biosynthesis of the siderophore stapyloferrin B (SB). Heme-binding by SbnI reduces SB biosynthesis. We are investigating the mechanism of the heme relay from IsdB to SbnI to regulate SB production. First, we present a crystal structure of a complex between human Hb and IsdB. In this complex, the asubunits of Hb are refolded with the heme displaced to the interface with IsdB. We also observe that atypical residues of Hb, His58 and His89 of aHb, coordinate to the heme iron, which is poised for transfer into the heme-binding pocket of IsdB. Moreover, the porphyrin ring interacts with IsdB residues Tyr440 and Tyr444. Previously, Tyr440 was observed to coordinate heme-iron in an IsdB-heme complex structure. A Y440F/Y444F IsdB variant we produced was defective in heme transfer, yet formed a stable complex with Hb ($K_d = 6 \pm 2$ μM) in solution with spectroscopic features of the bis-His species observed in the crystal structure. From the kinetics of heme transfer and an analysis of crystal structures of IsdA and IsdB, we have developed a model of heme transfer between the heme binding NEAT domains of these cell wall anchored proteins. After transport of the heme by the ABC transporter IsdEF, heme is presumed to be captured by IsdG, an oxidase that degrades the porphyrin ring to release iron. We show by heme transfer kinetics, that in addition to heme degradation, IsdG can actively transfer heme to SbnI. Determination of the crystal structure of SbnI revealed homology to a family of free serine kinases that produce O-phosophoserine, a precursor of SB. Biochemically studies show SbnI is an ATP dependent free serine kinase. Inspection of the structure and docking experiments, suggest heme binds at a site near the C-terminus distinct from the kinase active site and this model is supported by site-directed mutagenesis. Together, our data supports a model of a heme relay from Hb through to SbnI to repress the production of the SB.

## SESSION X: Novel Therapeutics and Bacterial Chemosensation
***Sponsored by Manitoba Chemosensory Biology Group***
**Wednesday, June 20th, 2018**
**10:45 AM – 12:15 PM**

**IS 11**
**The Natural History of Antibiotic Resistance**
Gerard WRIGHT, McMaster University

The evidence is indisputable that resistance that tracks directly with antibiotic use. This phenomenon is a result of a failure to understand that antibiotics and resistance are the products of, and subject to, natural selection. Understanding the origins of antibiotics and their chemistries, the co-evolution of resistance and the chemical ecology antimicrobial substances is critical to grappling with the current antibiotic crisis and informing on new therapeutic options for the 21st Century. Essential to this understanding is an accurate survey of molecular mechanisms of antibiotic resistance throughout microbial communities. Some of our efforts to explore this chemical and genetic diversity will be described, with particular attention to the glycopeptide class of antibiotics

**SS10-1**
**Anti-Virulence Phage Therapy**
JG MCCUTCHEON, University of Alberta, DL PETERS[1], CM DAVIS[1], Jonathan DENNIS[1], [1]University of Alberta

Mounting antibiotic resistance exhibited by pathogenic bacteria threatens to erode our current clinical capabilities, and recent estimates suggest that by year 2050 antimicrobial resistance (AMR) will kill more people than cancer (10 million/yr.) and have cost the global economy 100 trillion dollars. One potential alternative treatment for highly antibiotic resistant bacteria is "phage therapy" (PT), the clinical application of bacteriophages to eradicate target bacteria. Several recent highly publicized cases in the US have shown that PT can be effective at saving patient's lives where antibiotics have failed. The effectiveness of phages towards their hosts greatly relies upon phages having the ability to recognize and bind to the bacterial cell's surface receptor. Identifying receptors, and phage receptor binding proteins (RBPs), will define the mechanism of phage-host cell interactions needed for efficient cell lysis. If host cells acquire mutations that result in the loss of phage surface receptors, the bacteria become resistance to phage predation and lysis. In most instances, resistance can be overcome by including multiple phages in a single preparation, or "cocktail", each with a different receptor or route of entry. Mutations to resistance against one phage have no effect on a different phage attacking through a different receptor. Alternatively, phages adhering to bacterial surface structures involved in virulence elaboration (or antibiotic resistance), create a conundrum for the bacterium. Mutation to resist phage binding or uptake through the loss of a virulence-related surface structure creates a bacterial cell less fit to survive in vivo, or create disease. This particular class of phages not only kills target bacteria, but also exerts selective evolutionary pressure such that escape mutants become less virulent or more sensitive to antibiotics, and potentially non-toxic. We have identified two broad host range phages (DLP1 & DLP2) capable of killing both *Stenotrophomonas maltophilia* (Sm) and *Pseudomonas aeruginosa* (Pa) strains. Screening a Pa transposon insertion library for phage resistant mutants showed that DLP1/2 infect cells through initial attachment to type IV pili as their primary receptor. Type IV pili are virulence factors on the bacterial cell exterior involved in attachment to surfaces, biofilm formation, and twitching motility. Clean deletion of the major pilin subunit encoded by *pilA* in Sm D1585 prevents infection by both phages, and complementation with the endogenous *pilA* gene restores infection. Similar work with minor pilin *pilE* confirmed this association. Mutation analysis of pili retraction motors shows that pilus retraction is required for successful phage infection. Surprisingly, DLP1/2 exhibit unique host ranges, suggesting different binding sites within the type IV pilus. Future work includes characterizing receptor: phage RBP interactions, examining therapeutic efficacy, and ascertaining phage resistance levels in vivo.

**SS10-2**

**Targeted method for control of *Campylobacter jejuni* and *Salmonella enterica* in broilers using single domain antibody-based approach**

Dea SHAHINAS, AbCelex Technologies Inc., ME ORTIZ[2], H PARSA[1], S BABAEI[1], M TEREBIZNIK[2], [1]AbCelex Technologies Inc., [2]University of Toronto at Scarborough

**Background:** The expansive market for broiler chickens, the emergence of antibiotic resistant strains of *Campylobacter jejuni* and *Salmonella enterica* and the antibiotic use ban in most regions necessitate novel control strategies. We propose the use of targeted single domain antibodies (sdAbs) to reduce the colonization load of *C. jejuni* and *S. enterica* in broiler chickens. **Hypothesis:** sdAbs, which constitute the structural and functional antigen-binding fragment of camelid antibodies, targeted to abundant and accessible targets of *C. jejuni* and *S. enterica* effectively neutralize the bacteria and inhibit their exponential growth in the intestine of market age broiler chickens. **Methods: 1)**Genomics and proteomics methods were employed to discover conserved, abundant and surface accessible targets. **2)** Phage display libraries were generated from the serum of immunized llamas/alpacas. Standard ELISAs were performed to pan and screen the libraries as well as to identify antigen-specific sdAbs; **3)** Next generation sequencing technology was employed to recover the full diversity of phage displayed sdAbs **4)** Target specificity was further confirmed with Far Western and immunoprecipitation studies followed up by mass-spectrometry identification; **5)** Gentamicin protection assays were used to analyze lead sdAbs' and engineered sdAbs' susceptibility *in vitro* and *ex vivo;* **6)** Oral gavage chick challenge as well as market-age broiler bird modelswith*C. jejuni* or *S. enterica* were used to test lead sdAbs' efficacy *in vivo.* **Results:** Three lead sdAbs inhibited *S. enterica* motility and two lead sdAbs inhibited *C. jejuni* motility. The anti-Salmonella sdAbsshowed a minimum inhibitory concentration (MIC) of 500µg/mL in the *ex vivo* gentamicin protection assay. Immunoprecipitation and Far Western studies confirmed target protein binding specificity based on mass-spec ID. Unlike anti-*C. jejuni* sdAbs that inhibit the motility which is essential for pervasion of the chicken intestine, anti-*S. enterica* sdAbs interfere with bacterial attachment and/or invasion of the host cell. sdAb engineering and expression system optimization improved the efficacy of the lead sdAbs in the *in vivo* oral gavage broiler young chick and market age bird- challenge model from 0.5-$\log_{10}$ to 2-$\log_{10}$ ($p<0.05$) reduction in colonization. **Conclusions:** sdAbs offer an effective strategy in reducing *C. jejuni* and *S. enterica* colonization in broiler chickens and pose a commercially viable means for food safety management in commercial broiler production.

**SS10-3**

**CDRD/LifeArc/Dstl - A novel Collaboration for Antibacterial Drug Discovery**

Sophie YURIST-DOUTSCH, , H ATKINS[2], P IRELAND[2], H BULLIFENT[2], K BIRCHALL[3], P COOMBES[3], P CANNING[3], C KETTLEBOROUGH[3], A SCOTT[2], E DULLAGHAN[1], [1]Centre for Drug Research and Development, [2]Defence Science and Technology Laboratory UK, [3]LifeArc formerly MRC Technology

Antibiotic resistance is a major worldwide health problem that is threatening our ability to treat common infectious diseases. A growing list of infections – such as pneumonia, tuberculosis, sepsis and gonorrhoea – are becoming harder, and sometimes impossible, to treat as antibiotics become less effective. Consequently, there is a dire need for the development of new therapeutics with novel mechanisms of action against unexploited targets. The majority of antibiotics, both approved and in late stages of development, are derivatives of old chemical classes, which hit targets for which underlying resistance mechanisms are already well-established. The Centre for Drug Research and Development (CDRD), Canada's national drug development and commercialization center, has joined forces with LifeArc (formally MRCT technology) and Dstl (Defence Science and Technology Laboratory, UK) to collaborate and identify antibacterial drug targets using a novel approach that capitalizes on commonality across pathogens. A transposon-based approach was used to identify genes essential for viability or virulence in multiple pathogenic bacteria. The initial list of genes was filtered based on computational and experimental criteria to select those with the highest potential to be anti-bacterial targets. This approach is currently being exploited to select targets to move forward into drug discovery programs. We have assembled a multidisciplinary team of microbiologists, medicinal chemists and bioinformaticians to work together effectively to discover and develop novel antibacterial drugs. The

team brings a unique perspective to this challenge and CDRD along with our partners LifeArc and Dstl, help create an effective clinical path forward.

## SESSION XI: Fungal Genomics and Diseases
## Wednesday, June 20th, 2018
## 10:45 AM – 12:15 PM

**IS 12**

**Lessons from high-throughput functional genomics analyses in *Neurospora crassa***

Katherine BORKOVICH, University of California, Riverside, AJC ALEXANDER CARRILLO[1], MJS MARKO SPASOJEVIC[1], IEC ILVA CABRERA[1], [1]University of California, Riverside

*Neurospora crassa* is the major model organism for filamentous fungi. Filamentous fungi are important for carbon recycling in the biosphere, as major plant pathogens and largely opportunistic animal pathogens, and as model systems for aspects of mammalian biology. *N. crassa* has a sequenced and annotated genome, 9,758 protein-coding genes and a nearly complete gene knockout mutant strain collection. Phenotypic data for 10 different growth or developmental attributes have been obtained for nearly 1300 mutants. Much of the data has been collected by undergraduates during summer programs or enrollment in research-based courses. Large groupings of mutants that have been analyzed include transcription factors, serine-threonine protein kinases, protein phosphatases and G protein coupled receptors. In many cases, chemical sensitivity or nutritional phenotypes are also available. We are employing statistical clustering analysis to assign mutants to different groupings based on their growth and developmental phenotypes. Results from RNAseq analysis and patterns of MAPK phosphorylation are being incorporated to confirm clustering relationships and to identify genes that are new members of identified or unknown pathways.

**SS11-1**

**Characterization of Fungal-Algal Interactions in Biological Soil Crusts Using Culture-Based and Metagenomic Approaches**

Steven HARRIS, University of Manitoba, E CARR[2], WR RIEKHOF[2], JR HERR[2], [1]University of Manitoba, [2]University of Nebraska

Biological soil crusts (BSCs) perform several critical functions in dryland ecosystems. BSCs are typically composed of cyanobacteria, bacteria, fungi, lichens, and mosses. Numerous studies are providing greater insight into the specific microbial species that inhabit BSCs from different geographic locations. Moreover, these studies are beginning to identify the functional traits associated with each species. An emerging theme is that whereas the specific composition of BSCs may not be conserved across locations, the functional attributes of BSCs are. Within this context, it becomes important to identify and characterize the functional interactions between each resident of specific BSC communities. Towards that end, we are seeking to characterize the species composition, functional traits, and interaction networks of different BSCs located in dryland ecosystems of Western Canada. Our specific focus is on the interactions between fungi and photoautotrophs (algae and cyanobacteria) in BSCs. Accordingly, we are using culture-based approaches to identify these microorganisms, followed by detailed phenotyping and systematic pairwise interaction experiments. Our culture-based analysis has to date identified 24 fungal (Chaetothyriales) and 43 algal isolates from eight distinct locations sampled within BSCs found in a semi-arid sand dune ecosystem (Jackman Flats Provincial Park, BC). The 24 fungal isolates exhibit a range of extremotolerant and interaction phenotypes. Parallel Illumina sequencing of ITS1 and 16S amplicons is also being used to comprehensively characterize the microbial composition of these BSCs. Current efforts are focused on integrating phylogenetic, phenotypic, and metagenomic data to understand the functional interactions that enable the formation and maintenance of BSCs.

**MGC 049**

**CRISPR-based functional genomic platforms for gene deletions and modulating gene expression in *Candida* pathogens**

Rebecca SHAPIRO, University of Guelph

Opportunistic *Candida* pathogens are the leading cause of fungal infections worldwide. Yet, comprehensive functional genomic analysis in many of these clinically important fungal pathogen remains cumbersome. This issue is further compounded when double mutant lines need to be generated for studying complex genetic interaction networks underpinning fungal pathogenesis. Here, we have developed a CRISPR-based toolkit for functional genomic analysis in *Candida* species, using canonical CRISPR-based mutations, as well as newer strategies focused on CRISPR-based regulation of gene expression. The first strategy is a CRISPR-Cas9-based 'gene drive' platform for rapid, precise, and efficient genome editing in *Candida albicans*, enabling applications for global genetic analysis of fungal pathogenesis. In our gene drive system, a modified DNA donor molecule is used that acts as a selfish genetic element, replaces the targeted site, and propagates to replace any additional wild-type locus it encounters. Coupling this approach with newly identified mating-competent haploid *C. albicans* lineages, we can rapidly and efficiently create diploid *C. albicans* strains that are double homozygous deletion mutants, enabling us to create large scale double-deletion libraries and analyze complex genetic interactions networks in *C. albicans* for the first time. We demonstrate the power of this technology by generating two double-gene deletion libraries, targeting factors involved in either drug efflux or cellular adhesion, identify central regulators of these pathways, and determine how genetic interaction networks shift under diverse environmental conditions. In addition to this, we have developed two powerful technologies, never previously used in fungal pathogens: CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), for applications in diverse *Candida* species. Both of these technologies rely on a nuclease-dead Cas9 (dCas9) that is fused with transcriptional repressors (for CRISPRi) or activators (CRISPRa), and is targeted to a specific promoter region by a guide RNA in order to repress or activate gene expression from a targeted locus. By generating these dCas9 constructs and designing unique guide RNAs, we demonstrate the ability of these systems to specifically repress or induce gene expression in two *Candida* species. Since, unlike classic CRISPR systems, these platforms do not require a DNA repair construct but simply a unique 20bp guiding sequence, the simplicity of this system lends itself to high-throughput strain generation. Using a highly-efficient, high-throughput Golden Gate cloning strategy, we are able to efficiently and rapidly generate large numbers of fungal mutant strains that over- or under-express any gene of interest, providing a powerful new tool for functional genomic analyses in fungal pathogens.

**SS11-3**

***Cryptococcus neoformans* genomics influence human immunologial responses**

Aleeza GERSTEIN, University of Manitoba, D.R. BOULWARE[2], D. MEYA [3], K. NIELSEN[2], [1]University of Manitoba, [2]University of Minnesota, [3]Makere University

Patient outcomes are due to a complex interplay between the quality of medical care, host immunity factors, and the infecting pathogen's characteristics. To probe the influence of pathogen genotype on human immune response, we examined *Cryptococcus neoformans* isolates collected as part of the Cryptococcal Optimal ART Timing (COAT) HIV trial in Uganda. An array of human immunological phenotypes including cytokine levels and disease parameters were measured on all patients. We whole genome sequenced 39 isolates from ST93, the most frequently observed sequence type (ST). We focused our analyses on the 574 variants that were variable among the genomes, were not associated with a centromere or telomere, and were predicted to have a fitness effect. Logistic regression tests identified 50 candidate genes associated with human immunological response. We infected mice with available gene knockout strains and found that ~25% directly influenced survival or fungal burden. This work demonstrates that fungal genotype can significantly influence immune responses and provides important candidate genes for future studies on virulence-associated traits in *Cryptococcus*.

## SESSION XII: Indigenous Health and Water Security
## Wednesday, June 20th, 2018
## 10:45 AM – 12:15 PM

**IS 13**
**Water Insecurity in Indigenous Communities: Beyond Physical Impacts**
Lalita BHARADWAJ, University of Saskatchewan

In Canada, a nation recognized for its natural wealth of fresh water supplies, the provision of safe drinking water is a pressing health issue confronting Indigenous communities. Many communities in Canada live with long-term drinking water advisories, high-risk drinking water systems and experience health status and water quality below that of the general population. A heightened understanding, gathering information from the voices of the communities, on the nature of existing drinking water issues and associated health related challenges is needed. Drinking water quality and supply are key challenges. Health consequences extend from the individual to community levels and beyond physical symptoms. Inadequate drinking water has widespread implications to the well-being of Indigenous people. A more holistic understanding of the relationship between drinking water challenges and Indigenous well-being is required to better inform policy decisions for drinking water provision.