Student Symposium Competition Abstracts

STUDENT SYMPOSIUM I
Tuesday, June 19, 2018
1:30-3:30 PM

Note: **AEM:** Applied Environmental Microbiology
II: Infection and Immunity | **MGCM:** Molecular Genetic Cellular Microbiology

AEM SSC 01

The impact of an active soil microbial community on greenhouse gas emissions in the Canadian high Arctic

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Climate warming and subsequent permafrost thaw allows the currently frozen carbon and nutrient stores to become available for metabolism by microbial communities. This can result in a positive feedback loop of greenhouse gas (GHG) soil emissions. Nitrous oxide (N2O) and methane (CH4) are two of the most important GHGs after carbon dioxide. Understanding the flux of these GHGs in the High Arctic and how this flux is related to the active microbial community is key for predicting future GHG emissions and climatic warming. We aimed to characterize the community of denitrifiers, nitrogen-fixing bacteria, and methanotrophs to determine the effect this portion of the microbial community has on the N2O and CH4 gas flux at an ice-wedge polygon (IWP) site in the Canadian high Arctic. Through a combination of Metatranscriptomics, qPCR, gas flux data, functional gene sequencing, and *in situ* stable isotope probing (SIP) we demonstrate a functional community of high affinity methanotrophs, denitrifiers, and nitrogen-fixing bacteria which are potentially responsible for a positive N2O flux and a negative CH4 flux at the High Arctic site. Metatranscriptome sequencing of soils overlaying permafrost identified the functional microbes currently present in two soil types at the site: the drier soil of the polygon interior and the wetter trough soil. We detected gene transcripts involved in nitrogen fixation, denitrification, and methanotrophy in both soils. QPCR indicated a higher abundance of denitrification (*nirS*) and methanotrophy (*pmoA*) genes in the trough soils compared to the polygon interior soils. This coincided with higher N2O emissions (0.29 $mg \bullet m^{-2} \bullet day^{-1}$) and higher CH4 uptake (-124.7 $mg \bullet m^{-2} \bullet day^{-1}$) in the trough compared to polygon interior soils. Functional gene sequencing of *nirS* gene and *nifH* gene also reflected the differences in the microbial community between the polygon interior and the trough soils. The denitrifying community was dominated by Rhodocyclaceae in trough soils, while the polygon soils were dominated by Rhizobiales and Acidiferrobacteraceae. The *nifH* containing microbial members in trough soils were dominated by Desulfobacterales, Rhizobiales, and Gallionellaceae, while in the polygon soils only the Rhizobiales were dominant. Finally, *in situ* SIP with C13 methane and subsequent *pmoA* sequencing demonstrated that all the labelled *pmoA* genes in the soils were related to high-affinity methanotrophs, suggesting this group of organisms is responsible for the methane sink. The active microbial community in the IWP soils is likely responsible for apparent N2O emissions and CH4 uptake in the High Arctic. The differences between the flux these gases at the wetter through soils and the drier polygon interior soils is mirrored in the differences of the dominant microbial community members and the relative abundance of functional genes responsible for these biogeochemical processes in these soils.

II SSC 05

Structure and peptidoglycan association of peptidoglycan *O*-acetyltransferase B from *Neisseria gonorrhoeae*

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The proper maintenance of the peptidoglycan (PG) sacculus is critical to cellular viability. PG is composed of repeating *N*-acetylglucosaminyl and *N*-acetylmuramoyl residues, the latter of which is often found to be decorated with an *O*-acetyl group on the C6-hydroxyl in pathogenic bacteria. O-Acetylation is a major virulence factor as it confers resistance to lysozyme of the innate immune system increasing pathogenicity. O-Acetylation has added importance in Gram-negative bacteria as it is also a means of control of lytic transglycosylases (LTs), endogenous enzymes whose activity would otherwise be autolytic. Gram-positive and Gram-negative bacteria both contain O-acetylated PG but each employ a unique enzymatic system. The former utilize *O*-acetyltransferase A (OatA), a bimodular *O*-acetyltransferase, while Gram-negative bacteria use PG *O*-acetyltransferases (Pat) A and B as well as *O*-acetylpeptidoglycan esterase. The additional esterase removes the modification allowing for LT activity, providing added control of the LTs. To gain a greater understanding of the system, the structure of PatB was determined at a resolution of 1.25 Å using x-ray crystallography. PatB adopts an atypical α/β hydrolase fold with a conserved catalytic triad (Ser125, Asp294 and His297). SGNH hydrolases also have a conserved Asn and Gly which stabilize the formation of the oxyanion hole. This holds true for homologs of Ape, but homologs of PatB contain a conserved Ser in place of Gly. We hypothesized that this change may help to differentiate esterase vs transferase activity, where the latter would need to exclude access of water to the acetyl-enzyme intermediate thereby facilitating efficient acetyl transfer to PG. The substitution of Ser to Ala results in a 2-fold increase in esterase activity and a 2-fold decrease in transferase activity *in vitro* suggesting that this residue may function to aid in precluding water from the active sites of transferases. Efficiency of the transferase reaction may also stem from the nature of the interaction between PatB and PG. PatB was found to strongly associate with PG despite lacking a carbohydrate binding motif or binding cleft. PatB contains two pairs of antiparallel β strands, one on either side of the active site which we have termed “wings”. These motifs were not found in OatA from *Streptococcus pneumoniae*, which is unable to bind PG, or in various structurally similar esterase’s identified by the Dali server. Phyre2 predictions for over 30 PatB homologs were also generated, all of which contained the wing motifs and residues that are predicted to be involved in PG binding, suggesting that the wings are likely a conserved structural feature of PatB. In this study, we present the first crystal structure of a Gram-negative PG *O*-acetyltransferase, PatB, which includes unique wing motifs. These motifs lend insight to this system as a whole as they may be involved in the spatial regulation of PatB and its specificity as a transferase.

MGCM SSC 09

CRISPRStudio: a user-friendly software for rapid CRISPR array visualization

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CRISPR-based typing is a powerful tool for bacterial strain tracking. Initially discovered in bacteria, CRISPR-Cas systems act as an adaptive defense mechanism against invading nucleic acids. CRISPR loci harbor a rapidly-evolving structure, caused by the integration of spacers, short DNA fragments originating from invading elements. Due to the hypervariable nature of the locus, CRISPR typing has been used to improve strain typing resolution for a variety of bacterial species in clinical, environmental, and industrial settings. Large-scale CRISPR typing studies require an efficient method to showcase CRISPR array similarities across multiple isolates and the best way is to graphically represent CRISPR arrays with colored shapes. However, no software is available to automatize the construction of such figure and the current method requires to manually convert each spacer to a color-coded shape, which makes it very tedious. To improve the graphical representation of CRISPR arrays, we developed CRISPRStudio, a user-friendly software generating CRISPR array publication-ready figures. CRISPRStudio is a python script divided in three simple commands. The first command consists of extracting individual spacers, by finding a regular expression pattern corresponding to the expected position of the spacers sequences in a CRISPRDetect output file. In order to assign a color for visualization, spacers must be aligned to determine which ones are homologous, and therefore have the same colors. The alignment result is fed to the second command, which clusters homologous pairs of spacers into groups and assigns two random hexadecimal (HEX) codes to each cluster. The two HEX codes will correspond to the square and the diamond colors. Finally, the third command takes a list of samples names, transforms their corresponding spacers to the color-coded format and writes a SVG file, which can be opened in graphics editor software. The SVG file shows each spacer displayed as a square with a diamond in its center. CRISPR arrays are shown on individual rows, respecting the 5' to 3' orientation and aligned right. To test CRISPRStudio, we used a previously described *Salmonella* dataset. We were able to display all CRISPRs from the 206 isolates under ten minutes, compared to approximately twelve hours with the manual method, representing a striking 72-fold increase in speed. In addition to improving efficiency, CRISPRStudio facilitates CRISPR visualization, with its two-color system which helps distinguishing different spacers. By aligning arrays on the right, spacers on the 3' end, which are more likely to be shared across the dataset, will be at the same vertical position, making similar spacers between samples easy to identify. Creating a SVG also makes it very simple to adjust figures on demand. Together, these results show the versatility of CRISPRStudio and its potential to support and greatly accelerate CRISPR array visualization in large-scale CRISPR typing studies.

AEM SSC 02

Application of culturomics to elucidate microbiome-inflammation interactions in the porcine intestinal tract

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Studies using next-generation sequencing methods to characterize the intestinal microbiome have increased substantially in recent years, but the elucidation of bacterial function has received less attention. A salient goal of our research team is the development of effective alternatives to antibiotics; the aim of the current study was to isolate, characterize, and store bacteria from the porcine intestinal tract ± inflammation to identify strains capable of site and tissue specific interactions. To recover candidate bacteria, 24-large white Landrace cross piglets were randomly separated into two groups and orally gavaged with Salmonella enterica Serotype Typhimurium DT104 or Columbia broth as a control. Ingesta and mucosa samples from the ileum, cecum, spiral and ascending colons were collected from live animals under anesthesia at 2, 6, and 10 days post-inoculation (i.e. four replicate animals per treatment-time combination). To isolate bacteria, strict anaerobic conditions using an array of methods were applied, including direct plating, a modified Ichip, long-term enrichments, and endospore germination techniques. This isolation strategy yielded a total 359 taxa spanning 80 genera and eight phyla (*Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Lentispiracea, Proteobacteria, Spirochaetes*, and *Synergistetes*). Of the isolated bacteria, 120 were potentially new taxa, with 65, 39 and 16 taxa representing new families (<92% similarity to identified bacteria in the Ribosomal Database Project), new genera (95-92% similarity), and new species (97-95% similarity), respectively. The greatest diversity of bacteria and largest number of new taxa were recovered using long-term enrichment strategies (Shannon-Weiner index [SW] of 3.47). The modified Ichip technique was the best method to isolate bacteria belonging to new families (24% of isolated taxa); however, diversity was reduced relative to enrichment methods (SW of 3.00). Surprisingly, the diversity of bacteria isolated using the direct plating method was greater than the Ichip (SW of 3.24). In contrast to recent reports, the lowest diversity of bacteria was recovered using endospore germination methods (SW of ≤2.17). Salmonella Typhimurium induced acute enteric inflammation in a time-dependent manner (i.e. acute, subacute and chronic salmonellosis), and a number of bacteria were differentially associated with inflamed mucosa including *Bacteroides fragilis, Christinellaminuta*, and *Lactobacillus salivarius*. An Enteric Bacterial Collection (EBaC) has been established at Agriculture and Agri-Food Canada Lethbridge, and representatives of all bacterial taxa collected from pigs (and other animals) have been accessioned into the collection. In conclusion, this study demonstrated that a comprehensive isolation strategy is required to generate a diverse collection of enteric bacteria for subsequent elucidation of function and to develop innovations for human and livestock medicine.

II SSC 06

Design and Evaluation of Tobramycin-Cyclam Conjugates as Classic Antibiotic Potentiators against Gram-negative bacteria

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Gram-negative bacteria are intrinsically resistant to many antibiotics and often difficult to treat because of their protective outer membrane (OM) and constitutively over expressed efflux mechanism. In 2017, the WHO designated carbapenem-resistant *Acinetobacter*, *Pseudomonas*, and various Enterobacteriaceae as the most critical group of the priority pathogens for which effective therapy is urgently needed. Aminoglycosides (AGs) are a unique class of Gram-negative-potent antibiotics because of their self-promoted uptake mechanism that is independent of porin channels. AGs perturb the OM of Gram-negative bacteria by displacing the stabilizing cross-bridging divalent cations in a concentration-dependent manner and traverse the inner membrane via an energy-dependent process. At lower concentrations, AGs interfere with the fidelity of ribosomal protein translation while they disrupt the inner membrane at high concentrations. Unfortunately, AGs exhibit nephrotoxicity and ototoxicity at high concentrations. We have shown that specific modification of a tobramycin scaffold and its systematic conjugation can decouple the membrane effects of this class of drugs from its ribosomal functions in *Pseudomonas aeruginosa*. *P. aeruginosa* infections are exceptionally difficult to treat due to their extremely low OM permeability (100 times lower than E. coli) and highly selective porin channels. Tobramycin is the most potent AG against *P. aeruginosa*. Tobramycin-Cyclam (tob-cyc) conjugates were therefore conceptually developed as an adjuvant to potentiate several classes of antimicrobial agents by disrupting the OM, i.e. the amphiphilic tobramycin domain of the conjugate permeabilizes the OM while the cyclam domain sequesters the divalent cation needed to maintain the integrity of the OM. Whereas the MIC of tobramycin by itself against wild-type *P. aeruginosa* PAO1 is 1 mg/ml, the MICs of the conjugates were found to be > 1024 mg/ml, indicating that the conjugates no longer bind to the ribosomes. An ideal adjuvant is a small molecule that is inactive by itself but potentiates a primary antibiotic when used in combination. Checkerboard studies were then used to assess the synergistic properties of tob-cyc conjugates in combination with other antibiotics. In the presence of low concentrations (2 – 16 mg/ml or < 10 mM) of the conjugates, the MICs of legacy antibiotics, particularly cephalosporins, were significantly reduced (> 32-fold potentiation) in wild-type, MDR, and XDR *P. aeruginosa*. *P. aeruginosa* was also sensitized to antibiotics that are otherwise inactive against it, such as minocycline, chloramphenicol, rifampicin, etc. Tobramycin alone could not potentiate these antibiotics, even at relatively higher concentration. Indeed, the newly developed tob-cyc conjugates are classic adjuvants that can rescue the efficacy of legacy antibiotics, thus expanding the usefulness and therapeutic space of existing therapies.

MGCM SSC 10

PsrA is a regulator of virulence and cyst biogenesis in *Legionella pneumophila*

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In its natural and urban anthropogenic aquatic environments, *Legionella pneumophila* is an intracellular parasite of protozoa. It is also an accidental pathogen that if aerosolized and inhaled by susceptible humans, can cause the pneumonia known as Legionnaire's disease, which occurs when the bacteria invade and replicate within lung macrophages. *L. pneumophila* has a broadly "biphasic" lifecycle, where the bacteria typically alternate between an intracellular replicative phase and a transmissive phase, the latter related to and driven by stationary phase processes. Transmissive phase is epitomized by a highly differentiated cyst form. Cysts are highly motile, and are morphologically distinct being irregular coccoid cells with thickened cell walls and multiple membrane laminations, and containning prominent cytoplasmic poly-3-hydroxybutyrate granules. These adaptations enable environmental persistence while between protozoan hosts. The cyst form is the virulent stage, optimized for uptake into new hosts. While significant advances have been made in establishing the framework of the regulatory cascade controlling cyst biogenesis, there remains much to understand in identifying and characterizing the pathways involved. Here we present the ongoing findings pertaining to the *L. pneumophila* orthologue of PsrA, a TetR-family transcriptional regulator that has a role in the cyst differentiation process. A *psrA* deletion strain was generated and assessed for growth and cellular morphology phenotypes *in vitro*, and *in vivo* in the natural protozoan host, *Acanthamoeba castellanii*. For comparison and control, the parental *L. pneumophila* Lp02 and *in-trans*complemented or overexpression strains bearing plasmid-borne *psrA* were included in experiments. Extensive morphological studies were conducted via differential interference contrast (DIC) microscopy, as well as transmission-electron microscopy to examine the ability of the strains to grow intracellularly and develop the distinct cyst morphology. In addition to a demonstrable defect in intracellular growth capability, *psrA* strains have titratable and distinct morphological phenotypes exhibited both in cyst formation, and *in-vivo* behaviour in the host cell itself. We are presently exploring the detailed genetic processes through which PsrA acts via transcriptomic analysis of the different *psrA* strains, to determine the genes through which PsrA is acting to mediate cyst formation and virulence processes. Taken together, our findings indicate the transcriptional regulator PsrA plays a substantial role in optimal virulence and cyst formation in *Legionella pneumophila.*

STUDENT SYMPOSIUM II
Wednesday, June 20, 2018
1:30 - 3:00 PM

AEM SSC 03

Quantification and Characterization of Vancomycin-Resistant Enterococci: A Comparison of Two Wastewater Treatment Processes

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Antimicrobial resistance (AMR) is an ever-growing public health problem that is threatening the sustainability of the global healthcare system. Wastewater treatment plants (WWTPs) are considered a possible point of control for the environmental spread of AMR. The objective of this study was to determine how biological aerated filter (BAF) and conventional activated sludge (CAS) treatment processes influenced the presence and characteristics of vancomycin-resistant enterococci (VRE). Culturing methods for *Enterococcus* and VRE combined with quantitative PCR for total bacteria, *van*A (vancomycin resistance) and class I integrons, were used to monitor primary (PE) and final effluents (FE) from the WWTPs over a 2-year period. Relationships between the removal of enterococci/VRE and chemical and environmental factors were examined using principle component analysis. *Enterococcus* spp. (n=1200) were isolated from the biomass, primary (PE) and final effluents (FE) of the WWTPs and screened for vancomycin resistance using broth-based susceptibility testing (MIC 4mg/L). Presumptive VRE isolates (n=313) were speciated (using *gro*EL loci) and underwent disc susceptibility testing for 12 antibiotics. Relationships among species, frequency of resistance, and sampling location were determined using the chi-square test and hamming distances. The BAF system removed more enterococci from the waste stream than the CAS system with reductions of 3.21 logs and 2.02 logs, respectively, which correlated with alkalinity and total suspended solids (TSS) content of the wastewater. VRE were removed at the same efficiency as the overall enterococci population in both WWTPs. The species composition of the PE isolates was similar for both WWTPs. The BAF FE group had a higher relative proportion of *E. faecalis*, while the CAS FE group had a higher relative proportion of *E. faecium* ($p=0.039$). The CAS FE group also contained more *E. casseliflavus* and *E. gallinarum* isolates ($p=0.049$) than the BAF FE group. A shift to a higher relative abundance of *E. faecium* in the CAS system, could account for increased nitrofurantoin resistance ($NTRO^R$, $p<0.001$), decreased quinupristin/dalfopristin resistance ($QUIN^R$, $p=0.003$) compared to the BAF system and a higher frequency of $NITRO^R$ ($p=0.012$) and lower frequency of $QUIN^R$ ($p=0.007$) and streptomycin resistance ($p=0.022$) in the CAS FE isolates as compared to BAF FE. Multidrug resistant isolates were common (45.7% to 62.4%) in both systems. In total, 73 unique AMR phenotypes were identified; with most AMR phenotypes being specific to either the PE or FE of each WWTP. Both WWTPs were effective at removal of enterococci and VRE from waste streams with the efficiency influenced by the alkalinity and TSS content of the wastewater, which should be investigated further. Prevalence of AMR phenotypes is indicative of the species composition of the wastewater. Future work includes whole genome sequencing and comparative genomics of selected isolates.

II SSC 07

AB_5 toxin-dependent inhibition of ganglioside-mimicking gut bacteria: a novel mechanism of bacterial competition

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Campylobacter jejuni is a significant cause of bacterial acute gastroenteritis worldwide. *C. jejuni* is also the leading cause of paralysis, eliciting Guillain-Barré syndrome in humans by mimicry of peripheral nerve gangliosides with its surface exposed lipooligosaccharides (LOS). In some cases, this mimicry leads to a breakdown in immune tolerance and results in self-reactive antibodies which damage the nerve cells. Interestingly, the commonly mimicked GM1 ganglioside is also the main receptor for cholera toxin (CT) from *Vibrio cholerae* as well as heat-labile enterotoxin (LT) from *Escherichia coli*. We have shown that these two toxins bind *C. jejuni* strains that mimic GM1 gangliosides and alter the permeability of the cell membrane resulting in reduced growth. However, when we used a strain of *E. coli* engineered to display this same mimic on its surface, no clearance was observed in agar growth assays consistent with no change in ethidium bromide influx in a permeability assay. *C. jejuni* NCTC11168 shows natural variation in its ability to mimic GM1 ganglioside. Due to phase-variation in the galactosyltransferase *cgtB*, the population of this strain always exists as a mixture of mimics and non-mimics. It was unclear why the strain retained this variation, given that mimicry of host gangliosides lends a significant fitness advantage by camouflaging the bacterium from host immune defenses. Analysis of isolated LOS and sequencing of genomic DNA from cells exposed to the toxins revealed that the resulting population no longer mimicked GM1 ganglioside due to a frameshift in the *cgtB* gene. This led to the hypothesis that *C. jejuni* 11168 may retain variation in the *cgtB* gene to avoid negative effects upon contact with these toxins in the host or the environment. Further investigation involving fluorescent microscopy of chicken cecal cross-sections identified additional resident bacteria that appear to mimic the antigen. When chickens were fed the toxins, a marked reduction was observed in some bacterial families after analysing bacterial populations by 16S rRNA sequencing. Our studies identify a new interaction between *C. jejuni* and toxin-producing pathogens that has not been previously described and suggests the presence of more ganglioside-mimicking bacteria that may alter our perception of bacterial competition in the gut.

MGCM SSC 11

FNR is a regulator of *Salmonella* pathogenicity island 2 in *Salmonella* Typhimurium

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Salmonella Typhimurium must accurately coordinate virulence gene expression in response to the wide variety of environments that it encounters. In the intracellular environment of host cells, *Salmonella* pathogenicity island 2 (SPI-2) is essential for survival and proliferation. However, studies have shown that SPI-2 is induced by an unknown signal prior to the invasion of epithelial cells in the gut lumen. Under static growth in a neutral pH minimal medium with a glycerol carbon source, supplemented with the alternative electron acceptors fumarate and trimethylamine *N*-oxide, RNA-seq revealed expression of SPI-2 was highly induced in a Δ*fnr* mutant. Under the same conditions, SPI-1 genes were unchanged when

compared to the WT. ChIP-seq revealed putative FNR binding sites at SPI-2 promoters including the major SPI-2 regulator *ssrAB*, and apparatus protein operon *ssaBCDE*. Proteomic analysis revealed the presence of abundant SseA and SifB SPI-2 effector proteins in the cytoplasm of Δ*fnr* cells, but not in the WT. The activity of FNR as a transcriptional regulator directly corresponds to the absence of oxygen, therefore O_2 can be considered a major signal for the activation of SPI-2. This study is the first to reveal genetic control of SPI-2 by FNR in *S*. Typhimurium. We propose that direct repression of SPI-2 by FNR occurs in low O_2 conditions and that upon detection of increased O_2, like at the host epithelial border, repression by FNR is lifted.

AEM SSC 04

Comammox bacteria are the dominant ammonia oxidizers in rotating biological contactors of a municipal wastewater treatment plant

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Wastewater treatment plants (WWTPs) aim to remove ammonia from wastewater to prevent eutrophication and toxicity to aquatic animals. Nitrification oxidizes ammonia to nitrate via nitrite in two enzymatic steps, historically thought to be mediated by distinct groups of microorganisms. Aerobic ammonia oxidation was thought to be conducted by only ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), and nitrite oxidation was thought to be carried out by nitrite-oxidizing bacteria (NOB). However, recent discoveries showed that certain species of *Nitrospira* are capable of catalyzing both steps of nitrification in the process of complete ammonia oxidation (comammox). Little is known about comammox bacterial abundance and diversity in WWTPs, and no studies have investigated comammox bacteria in tertiary wastewater treatment systems. This study investigated ammonia oxidizers in nitrifying rotating biological contactors (RBCs) at the Guelph WWTP. As water flows from RBC 1 to 8, the ammonia concentration decreases through microbial oxidation. Due to a predicted low ammonia adaptation of comammox bacteria, we hypothesized that the relative abundance of comammox bacteria would increase as ammonia concentrations decreased along the RBC flowpath. To test this, we used metagenomic sequencing of RBC biofilm samples to assess the relative abundance and diversity of comammox bacteria in this tertiary treatment system. DNA was extracted from RBCs 1 and 8 from one treatment train sampled three times in 2010, and from RBCs 1 and 8 from all four trains in 2016. Profile Hidden Markov Models (HMMs) for taxonomic markers and functional genes were used to quantify the relative abundances and taxonomic affiliations of nitrifiers from unassembled metagenomic reads using MetAnnotate. Further quality control, assembly, annotation, and binning of metagenome reads was performed with the ATLAS pipeline. Taxonomic profiling of all microorganisms revealed that *Nitrospira* spp. dominated the RBC microbial community. The HMM hits for the ammonia monooxygenase subunit A (*amoA*) gene demonstrated that comammox-associated *Nitrospira* sequences represented the most abundant group of ammonia-oxidizing microorganisms. Contrary to our initial hypothesis, the relative abundance of comammox bacteria was higher in RBC 1 metagenomes than RBC 8 metagenomes. We recovered highly complete metagenome bins that classified to the genus *Nitrospira*. Several individual bins contained contigs with the genes for complete ammonia oxidation (i.e., *amoA, hao, nxrB*). Phylogenetic analysis revealed that multiple comammox bacterial taxa coexist in this system, several of which are novel. These RBCs represent large and stable biofilm systems for studying the ecology of comammox bacteria. This work represents an important step toward understanding microbial contributions to nitrification in these systems and may eventually help guide operational practices for improved effluent quality.

II SSC 08

Decoding Bad Bugs: Predicting antibiotic resistance phenotypes from genotype

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Diagnosing antimicrobial resistance (AMR) in the clinic is based on empirical evidence, which often leads to prescription of broad-spectrum antibiotics instead of targeted narrow-spectrum treatment. However, bacteria share AMR genes, leading to creation of strains increasingly resistant to first-line treatment options. As a result, less effective antibiotics are being used to replace currently recommended treatments. The slow turnaround time of culture-based diagnostics and misuse of antibiotics highlights the need for fast and accurate approaches to diagnose bacterial infections and determine antimicrobial susceptibility. The integrated use of whole-genome sequencing and bioinformatics resources has the potential to become the gold-standard method for diagnosing bacterial infections. Our current ability to computationally predict lab-observed AMR for *Escherichia coli* and *Pseudomonas aeruginosa* using the Comprehensive Antibiotic Resistance Database (CARD, card.mcmaster.ca), Resistance Gene Identifier (RGI), and Efflux Pump Identifier (EPI) was stronger for some antibiotic resistance phenotypes and weaker for others. These bioinformatics resources only include information published in peer-reviewed literature with clear experimental evidence of elevated resistance and filling gaps in published knowledge by performing new experiments improved some antibiotic resistance phenotype predictions. Specifically, we determined antibiotic susceptibility profiles for known resistance genes and identified new mutations potentially responsible for resistance phenotypes using the Antibiotic Resistance Platform and breseq, respectively. Determining the substrate profiles of *CMY-2* and *CTX-M-15* improved ceftriaxone, cefazolin, and cefixime resistance phenotype prediction, while numerous mutations across a variety of resistance mechanisms were predicted to be responsible for resistance to a variety of additional antibiotics, such as gentamicin, nitrofurantoin, ciprofloxacin, and trimethoprim-sulfamethoxazole. We are currently engineering the genomes of these two species to test the predicted genotype-phenotype relationship. Overall, our results demonstrate that screening gaps within the literature and underappreciated mutation limits our ability to predict *E. coli* and *P. aeruginosa* antibiotic resistance phenotypes computationally and the use of bioinformatics and experiments synergistically can improve the prediction of antibiotic resistance phenotypes. The development of timely and accurate genotypic approaches to predict antibiotic resistance phenotypes promotes the improvement of clinical outcomes and antibiotic stewardship.

MGCM SSC 12

Genotype-environment interactions lead to differential pigmentation among strains of human pathogenic yeast *Cryptococcus neoformans*

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Melanin pigments, polymers of oxidized substrates with a characteristic black/dark brown color, are ubiquitous in nature and are produced by many organisms ranging from microbes to humans. In the pathogenic yeast *Cryptococcus neoformans* species complex (CNSC), melanin pigments act as a radical scavenger resulting in the removal of harmful oxygen and nitrogen radicals from the cellular environment. CNSC is an opportunistic environmental pathogen that causes skin lesions, lung infections and fungal meningitis in immunocompromised individuals. Mutant CNSC strains whose melanin production has been eliminated are avirulent in mice. Melanin biosynthesis in CNSC is catalyzed by *LAC1*, a diphenol oxidase. We have observed melanin production to vary between strains with the yeast colonies ranging in color from white to increasingly darker shades of brown. A previous study identified five genomic regions including *LAC1* that contributed to melanin variance among laboratory-derived CNSC strains. However, specific genetic polymorphisms that might translate to phenotypic diversity were not identified. It is also unclear if and to what extent environmental factors affect melanin variance. The objectives of the current study were to quantify the variation in melanin production among a natural population of *C. neoformans* var. *neoformans* and to assess the contributions of genetic, environmental and genotype-environment interaction factors to such phenotypic variance. Using a spot densitometry assay on solid caffeic acid agar, we quantified the melanin production of 54 *C. neoformans* var. *neoformans* strains originated from 9 countries in three continents to three common stressors encountered during an infection, namely thermal, oxidative and nitrosative stresses. We Sanger sequenced seven nuclear loci to assess the genetic diversity of the test population. We also sequenced the complete promoter and coding regions of *LAC1* in all strains. Our results revealed extensive between-strain variation in melanin production in all tested conditions. Melanin production was significantly suppressed in thermal and oxidative stresses but was induced in moderate nitrosative stress. Over 50% of the melanin variance was attributable to genetic differences among the strains. A single nucleotide polymorphism in *LAC1* that causes an amino acid from in the putative catalytic domain of the LAC1 protein was identified to be significantly correlated with melanin levels. Genotype-environment interactions were a significant contributor to melanin variance at 28%, 29%, and 43% in thermal, nitrosative and oxidative stresses respectively. In contrast, environmental stress contributed relatively little to the total variance, ranging from 5-15%. While ourresults illustrate the complex interplay of genetic and environmental factors that leads to extensive phenotypic diversity in CNSC, further research is needed to fully elucidate their implications on the virulence of CNSC strains.

POSTDOCTORAL RESEARCH SYMPOSIUM
Thursday, June 21st, 2018
1:30 - 3:00 PM

PDR2

Development of microbial life detection technologies for future astrobiology missions

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Astrobiology and the search for life in our solar system is a major focus of space exploration. Astrobiology investigations are primarily focused on Mars, Europa and Enceladus, which are characterized by extremely cold temperatures. Life detection instruments currently available for astrobiology space missions are focused on identification of habitable environments or on detection of biosignatures (amino acids, small organic compounds), which can also be formed abiotically. The overall goal of our research is the eventual development of a small, portable, low cost, and low energy life detection platform that could be incorporated into future astrobiology missions. The platform would include the detection and sequencing of nucleic acids, unambiguous biosignatures. Given that life in our solar system is very likely to be microbial in nature, the focus of this project is the testing and optimization of pre-existing, automated, and miniaturized robust instruments for microbial nucleic acid extraction, library preparation, and sequencing. Applying these instruments in a novel astrobiology context, we have tested them both in the laboratory and at extreme cryoenvironment analogue sites in the Canadian high Arctic, including permafrost and cold spring systems analogous to Mars, Europa and Enceladus. Using an automated sample preparation unit (SPU) with high flight technology readiness (TRL-6), we successfully extracted nucleic acids in the laboratory from analogue samples containing low microbial biomass (10^5). The SPU is a sonication-based cell lysis instrument which generates cell lysates. These lysates were then successfully sequenced using the very small, light, and portable nanopore-based MinION sequencer from Oxford Nanopore Technologies. Lysates were prepared and sequenced in both crude extract form with minimal post-processing following SPU extraction (5 µm filtering) and in fully DNA purified form using a prevalent manual DNA extraction kit (Qiagen PowerlyserPowersoil) following initial extraction with SPU. Similarities were observed in the microbial profiles obtained using both types of extracts indicating a potential for the SPU and MinION to extract and sequence DNA with minimal processing and handling. Additional automated extraction systems will be tested using low biomass analogue samples to determine the ideal candidate providing extracts with the highest DNA concentrations and best sequencing potential. Testing of the newly available automated library preparation device, the VolTRAX (Oxford Nanopore), using analogue samples is ongoing. In addition to laboratory testing, we have successfully MinION sequenced DNA from analogue samples in situ at the McGill Research Station in the Canadian high Arctic and performed offline local basecalling using MinKNOW (v1.7.7). Furthermore, we have preliminarily determined the lower limit of minimum input DNA required by the MinION for successful sequencing (0.6 ng) and continue to push the system. A fully developed and optimized sequencing based micro life detection system could eventually be robotized and integrated into future planetary exploration space missions attached to surface rover platforms and/or penetrators.

PDR3

Characterization of the *Citrobacter rodentium* Cpx envelope stress response and its role in host infection

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In order to infect their hosts, Gram-negative pathogens employ a multitude of envelope-localized proteins, including adhesins and secretion systems. Envelope stress responses including the Cpx response allow bacteria to monitor and mitigate problems with the folding of these crucial envelope proteins. Previous studies have shown that the Cpx response is required for virulence of numerous pathogens, including *Citrobacter rodentium*, which causes transmissible murine colonic hyperplasia and is frequently used to model infections with the human-specific pathogens enteropathogenic *Escherichia coli* and enterohemorrhagic *E. coli*. However, the mechanisms by which the Cpx response promotes host infection by *C. rodentium* were previously unknown. In this study, we characterized the *C. rodentium* Cpx regulon in order to identify Cpx-regulated genes that are required for host infection. Using both transcriptomic and proteomic approaches, we found that the *C. rodentium* Cpx response upregulates expression of envelope-localized proteases and chaperones, while downregulating expression of fimbrial genes and type III secretion effectors. Mouse infections with *C. rodentium* strains lacking individual Cpx-regulated genes showed that both the chaperone/protease DegP and the disulfide bond oxidoreductase DsbA were essential for infection, but Cpx regulation of these two genes alone did not fully account for the impaired virulence of *ΔcpxRA*. Both deletion of *dsbA* and treatment with the reducing agent dithiothreitol activated the *C. rodentium* Cpx response, suggesting that it may sense disruption of envelope protein disulfide bonding. Our results highlight the importance of envelope protein folding in host infection by Gram-negative pathogens and suggest that the Cpx response plays a critical role in this process.

PDR5

Targeting a Peptide Transporter for Drug Delivery

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The role of ABC transporters for import of various metabolic substrates is exclusive to prokaryotes. This characteristic could be exploited in a "Trojan horse strategy" for delivery of anti-tubercular compounds. In this study the proposed dipeptide binding domain (DppA) of an ABC importer from Mycobacterium tuberculosis was examined for its substrate binding character. The DppA-Mtb encoding gene, Rv3666c was cloned, expressed and purified for crystallography and biophysical assays. The obtained crystal diffracted at 1.5Å revealing a bound endogenous peptide (SVAA). Substrate binding was tested using intrinsic florescence (IF), thermal denaturation (TD) and surface plasmon resonance (SPR), revealing DppA-Mtb binds neutral tri and tetra-peptides not di-peptides. Tri-valine was able to bind DppA-Mtb with greater affinity than other molecules tested, K_D 2.77 nM. No interaction was observed with the *E. coli* DppA natural substrate, L-ala-L-ala despite sharing 81% amino acid identity. To identify novel small molecules that may bind to DppA-Mtb a 10,000 compound screen by TD was carried out, identifying 20 small molecules with TM $\geq$ 2°C confirmed by SPR. The best hit, SJ000207136 achieved a KD of 1.79 μM. A screen of existing antimicrobial drug classes was made by SPR, identifying levofloxacin and novobiocin as low micromolar binders. Overall DppA-Mtb may be re-annotated to reflect binding to tri-peptides while antimicrobials and small molecules with antimicrobial characteristics may utilize this protein as a transport mechanism into the cell.

PDR1

Spatial competition in a model multispecies biofilms is mediated by extracellular matrix interactions and determines community morphology

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Bacterial communities in nature consist of hundreds to thousands of different organisms interacting with each other and the environment. Individual interactions between organisms strongly influence the composition of the community. In the natural environment, bacteria mainly grow as biofilms, which are assemblages of cells living within a self-secreted matrix at the interface between two phases. While competition for growth substrates is important, in biofilms, space to grow also becomes an important 'resource'. Natural communities contain so many different organisms that understanding individual interactions becomes too complex. Instead, we work with a model 3-species community, consisting of *Pseudomonas aeruginosa* PA01, *P. protegens* Pf-5 and *Klebsiella pneumoniae* KP-1 and have used it now to investigate spatial competition in colony biofilms. Colony biofilms are grown on an agar surface and as the biofilm expands from the initial inoculation point, strains are put into competition for space and nutrients at the leading edges. We have used strains chromosomally tagged to constitutively express fluorescent proteins and widefield microscopy to image colony biofilms as they grow. Quantitative image analysis of single species colony biofilms has been previously used to define patterns indicative of cooperative or competitive interactions, and discover various environmental and ecological factors that influence these patterns. For the current work, we have used quantitative image analysis of colony biofilms taken at multiple time points to characterize the complex and dynamic patterns generated by culturing single, pair-wise and 3-species mixtures of strains from our model community. As these patterns indicate the distribution of organisms in space, we use the term 'community morphology' to describe the shapes formed in the colony biofilms. *P. aeruginosa* PA01 is known to secrete extracellular DNA (eDNA) and use its type IV pilus (TFP) to twitch across surfaces, while *K. pneumoniae* secretes anti-biofilm polysaccharides and KP-1 is a particularly mucoid strain. Previously, a small colony variant (SCV) and non-mucoid (NM) variant (both due to chromosomal mutations) of PA01 and KP-1, respectively, were isolated from flow cell biofilms. The wild-type and mutant strains were co-cultured as colony biofilms together, with each other, and with *P. protegens* Pf-5. Addition of DNAse to the agar, TFP mutation in the SCV and decreased extracellular polysaccharide secretion in the NM KP-1 strain, all which interfered with strain's extracellular matrix interactions, made them less able to compete for space and altered community morphology. Interestingly, pairwise interactions did not always predict the outcomes of 3-strain biofilms. Through this work we have defined community morphology as a useful tool for analyzing bacterial interactions and shown how biofilm matrix interactions mediate spatial competition.

PDR4

Visualizing the Role of Alpha Toxin in Real Time During *Staphylococcus aureus* Sepsis

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Sepsis is a clinical manifestation of the host's inflammatory reaction to bloodstream infections causing microvascular perfusion problems leading to multiple organ dysfunction. Clinically, one of the biggest problems during sepsis is the formation of large platelet aggregates in the vasculature of poorly understood etiology leading to thrombotic microangiopathy, vascular occlusion and multi-organ failure. Insight into *Staphylococcus aureus* infection-mediated vascular damage and multi-organ dysfunction is limited. Therefore, using intravital spinning-disk microscopy we visualized rapid dynamic intravascular events in the microcirculation following Staphylococcal a-toxin (AT a-hemolysin, Hla) intoxication or infection. Intravenous AT injection induced rapid platelet aggregation, forming dynamic micro-thrombi in all organs examined. While the aggregates were washed away from the lung and skin vasculature, they were retained in the liver sinusoids and glomeruli of the kidney causing multi-organ dysfunction. Platelet aggregation and subsequent tissue damage in the liver were prevented with the AT neutralizing antibody MEDI4893*. Acute *S. aureus* infection resulted in sequestration of most bacteria by liver macrophages. Platelets were recruited to these cells early in the infection in a complement-dependent manner, but with time, an aberrant and damaging AT-dependent thrombosis predominated in the liver. Similarly, prophylaxis or treatment with MEDI4893* did not reduce early beneficial platelet recruitment, but did significantly reduce hepatic platelet deposition and thrombocytopenia resulting in reduced liver damage. These results indicate that AT elicits a damaging platelet response that can be prevented with MEDI4893*. This may represent a promising molecule to prevent staphylococcal induced intravascular coagulation, poor tissue perfusion and organ dysfunction.

PDR6

A novel, non-immunity-mediated resistance to T6SS attack

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The type six secretion system (T6SS) is employed by many gram negative bacteria to compete with neighbouring microbes both in the environment and in the context of infection. Resembling a molecular spear, the T6SS machinery contracts to thrust its tip into neighbouring cells, thereby delivering attached effector proteins. These toxins can target a variety of different cellular processes in both eukaryotes and prokaryotes and T6SS+ bacteria protect themselves against self-killing by expressing immunity genes to each of their secreted effectors. *Vibrio cholerae*, the causative agent of cholera, employs its T6SS to deliver five known effector proteins. Here we characterise a cryptic effector of *V. cholerae*, describing its mechanism of attachment onto the T6SS for delivery and furthermore demonstrating that it selectively kills a relatively narrower spectrum of species in comparison with the other *V. cholerae* effectors. To elucidate this difference and the underlying mechanism of selectivity, we employed genome comparisons as well as the *E. coli* Keio collection of specific gene knockouts. We identified a concerted effort of multiple stress response pathways and a variety of downstream genes that are critical to *E. coli*'s survival. Indeed, deletion of individual components of this response results in *E. coli* becoming susceptible to killing, suggesting that every piece of armour must be in place for a successful defense. In summary, this work characterizes a cryptic T6SS effector and reveals its killing activity in a T6SS-delivery setting. Moreover, we demonstrate a novel, non-immunity-mediated resistance to T6SS attack.