Posters Competion Abstract

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

AEM PC 01
Microbial communities in stormwater management ponds along a vegetation gradient. James BIE, University of Ontario Institute of Technology, A.E. KIRKWOOD[1], [1]University of Ontario Institute of Technology

Stormwater management ponds (SMP) are important receiving-waters in urbanizing environments because they serve as both water quantity and quality control-features for municipalities. Although SMP represent a significant mediation step in stormwater fate, very little is known about the resident microbial communities, including their activities and role in contaminant fate. To address these knowledge gaps, we set out to characterize SMP microbial communities by documenting and assessing: (1) microbial community composition and activity; (2) fecal-bacterial prevalence; and (3) cyanobacterial abundance and toxin production. Data collected for all three research objectives were analyzed as a function of vegetation cover, water quality, and pre-/post-storm events.Bacterioplankton from water samples differed from sediment communities in community composition and richness. Non-metric multidimensional scaling ordinations for each sampling date revealed unique bacterioplankton communities at the phyla-level for all study ponds. The dominant phyla in all SMP communities were Proteobacteria and Bacteroidetes. For one of the reference ponds, cyanobacteria was the dominant group, representing 45% of community composition. Redundancy analysis revealed significant gradients in conductivity, chloride, and nutrients, and clear similarities in water quality among 2 of the 3 reference ponds. There were no notable associations detected between community composition and water quality variables. Indicator fecal bacteria such as *E. coli* represented a very small portion of the total SMP bacterioplankton community (1-3%). *E. coli* density was low for most ponds over most dates, but certain SMP had consistently higher densities over the entire study period. This suggests a persistent source of *E. coli* in the sub-watershed draining into these SMP. In contrast, the reference ponds consistently had either very low or below detection levels of *E. coli*. It is important to note that *E. coli* density increased in all ponds after rain events. With respect to microcystin-LR production, most ponds had marginal non-detect levels, but a few ponds did test positive for microcystin-LR. Overall, our findings reveal that SMP bacterial communities are unique rather than similar to one another, with no clear associations with water quality at this early stage of analysis. This finding was unexpected considering the engineered nature of SMP and the fact that all study ponds are located in the same physiographic and climatic region. This likely reflects the variable hydrological conditions of SMP. From a management perspective, our results seem to indicate SMP are not significant reservoirs of fecal bacteria or the microcystin-LR toxin.

AEM PC 02
Metagenomic and functional analysis of Lake Erie's winter microbial communities. Sabateeshan MATHAVARAJAH, Trent University, M.X XENOPOULOS[1], D.W WALSH[2], [1]Trent University, [2]Concordia University

Microbial communities and related processes are thought to be dormant under the ice due to cold temperature and low light level, in many northern lakes, such as Lake Erie. However, under-ice phytoplankton blooms are common in Lake Erie, especially in the productive western basin, and are likely fuelling a diverse array of microbial metabolism that are important to carbon and nutrient cycling. To investigate the under-ice, ice-

associated and the nutrient cycling affecting microbial communities of Lake Erie, we initiated a comparative metagenomics study. To do so, 23 microbial samples were collected in February 2016 from stations located throughout Lake Erie. Metagenomic data was generated for 9 representative samples. Shifts in the proportion of major bacterial phyla occurred with respect to the basin's productivity gradient. Virus taxa dominated with abundance of related-genes corresponding to total dissolved phosphorus, relating phosphorus as a limiting constituent for virus morphology. Moreover, gene abundance varied across the samples in relation to total dissolved nitrogen and phosphorus, corresponding to related genes in assimilation pathways. Moreover, significant relationships were seen between varying nutrients of dissolved inorganic carbon, nitrate, and total phosphorus, in relation to the gene abundance of varying metabolisms. Through functional annotation of the metagenomics data, functional genes associated with these lineages are expected to shed light on the metabolic diversity of winter taxa.

AEM PC 03

The role of seed endophytes in the Plant Microbiome. Patricia MILLER, University of Toronto at Scarborough, RR FULTHORPE[1], [1]University of Toronto at Scarborough

Plants have a complex microbiome that includes both bacteria and fungi. Bacterial endophytes are important members of the plant holobiont as they positively influence plant growth and responses to pathogens and abiotic stresses. Plant microbiome research is a newly developing field with exciting possibilities and many unanswered questions. A pressing and current question remains about the source of bacterial endophytes. Studies suggest that plants derive their bacterial endophytic communities from the soil, the atmosphere, and the seed endosphere. The relative contribution of these sources has not yet been assessed. If seeds, however, determine the endophytic content of adult plants, then their origin is of importance to agricultural practices. We compared seed endophytes of various food plants, comparing the content of "organic" versus conventional seed sources. The endophytic populations of stems, roots, and leaves were compared to those of the seeds for a subset of these plants grown under laboratory and field conditions, using culture dependent and independent methods. Preliminary results suggest that plants have a high diversity of endophytes throughout and that seed endophytes are also found in other plant parts. Endophytic community comparison of organic versus inorganic seeds, leaves, and stems are also being compared, with results in progress.

AEM PC 04

Development of multiplex protocols for the detection of pathogenic fungi affecting grapevine woody tissues

Koyomi OZAKI, Armand-Frappier Institute - INRS, C. PROVOST[1], C. GUERTIN[2], É. DÉZIEL[2], [1]Centre de recherche agroalimentaire de Mirabel, [2]Armand-Frappier Institute – INRS

A wide variety of pathogenic fungi responsible for grapevine wood diseases can cause diverse symptoms in different parts of the plant. Several factors, such as the planting of new grapevines, cultural management practices and recent climate changes, may contribute to the spread of pathogenic fungi and diseases in vineyards. The standard laboratory methods generally used for the detection of fungi responsible for grapevine wood diseases are typically based on culture and phenotypic characterization. The development of techniques that are sensitive and specific, ideally not culture-based, to detect the fungal species present in Quebec and other provinces of Canada is desirable. The main objective of this project is therefore to develop laboratory methods to detect rapidly and simultaneously ten pathogenic fungi associated with five grapevine wood diseases that are or are expected to become, prevalent in Canada. The diseases targeted in the project are Esca, black foot, black dead arm, Eutypa dieback, and dead-arm. To achieve this goal, we chose the approach by multiplex real-time PCR, in which TaqMan® hybridization probes can target and bind to

different genomic markers of the pathogenic agents. We have selected to develop four multiplex systems, basically one for each disease, with three now ready to test field samples. The first duplex system, targeting the internal transcribed spacer 1 (ITS1) region of the studied pathogens, allow detecting DNA from *Phaemoniella chlamydospora* and *Phaoacremonium aleophilum*, which are two fungal species causing esca. ITS1 region refers to the spacer DNA located between 18S and 5.8S rRNA genes in fungi, and it is part of the ITS region, which is frequently used for the accurate identification of fungal species. The triplex system targets the H3 histone gene from 3 fungi associated with black foot disease: *Ilyonectria lirodendri*, *Ilyonectria macrodidyma*, and *Cylindrocarpon pauciseptatum*. Another triplex system is targeting the internal transcribed spacer 2 (ITS2) region, which is placed between 5.8S and 28S rRNA genes, was designed to detect the presence of three fungi of black dead arm: *Botryosphaeria dothidea*, *Diplodia seriata*, and *Neofusicoccum parvum*. The last duplex system, which will be used to detect two species responsible for Eutypa dieback and dead-arm disease, is currently under development. Once the development of 4 multiplex systems is complete, this tool could be used by the phytoprotection diagnostic laboratory at MAPAQ (Quebec's Ministry of Agriculture, Fisheries, and Food) to provide fast and reliable diagnosis of fungal infection in grapevine samples. Ultimately it should improve the health of all vineyards in Quebec, and would eventually be useful for the management of diseases in other vineyards in Canada.

AEM PC 05

Comparing Chiral Catalysts: Characterizing Epoxide Hydrolase Regio- and Enantioselectivity. Julia SZUSZ, Wilfrid Laurier University, GP HORSMAN[1], MD SUITS[1], [1]Wilfrid Laurier University

Epoxide hydrolases are an enzyme class with great potential in biocatalysis. Their regioselective and enantioselective properties can produce enantiopure fine chemicals in a more environmentally and fiscally responsible manner than current synthetic methods. In this work, epoxide hydrolases from *Streptomyces ghanaensis* (SghF) and *Micromonospora echinofusca* (MecF) are functionally and structurally characterized and compared to gain insight into the determinants of their opposing regioselectivity. We have solved the structure of the inverting hydrolase SghF including mutants and complexes to a resolution of 1.7 Å. Variants with mutations corresponding to the catalytic residues of SghF (D176N, H364A, H364Q and D337N) were created to evaluate their roles in the canonical two-step mechanism. Genome mining identified MecF as a proposed retaining hydrolase with high sequence similarity to SghF. The retaining activity was confirmed by chiral HPLC. This data can provide some insight into the regioselectivity of these enzymes and provide potential structural targets to improve enantioselectivity for use in biocatalysis.

AEM PC 06

Exploring the functional potential of seasonally anoxic boreal shield lake Archaean ocean analogues. Jackson TSUJI, University of Waterloo, S.L. SCHIFF[1], L. WU[1], J.J. VENKITESWARAN[2], L. MOLOT[3], R.J. ELGOOD[1], M.J. PATERSON[4], J.D. NEUFELD[1], [1]University of Waterloo, [2]Wilfrid Laurier University, [3]York University, [4]International Institute for Sustainable Development Experimental Lakes Area

The early Earth ocean of the Archaean Eon (≥2.5 billion years ago) is thought to have been anoxic, with high levels of dissolved iron and low levels of sulfur. Modern lakes have been identified with similar ferruginous (high iron and low sulfur) conditions in their permanent anoxic zones and have been used to explore the biogeochemistry of the Archaean ocean in a unique, whole-ecosystem manner. Study of such lakes, termed Archaean ocean analogues, has informed debate on the deposition of banded iron formations by identifying photosynthetic, iron-oxidizing bacteria (photoferrotrophs), related to the type strain *Chlorobium ferrooxidans*, as potential players in ancient microbial iron oxidation. However, a major limitation to the use of Archaean ocean analogues is their natural rarity; only four have been studied to date. Boreal shield lakes number in the millions globally and develop ferruginous anoxic waters. Unlike existing analogues, most

boreal lakes reoxygenate each spring and fall, regularly disrupting anaerobic processes. We investigated the possibility of using boreal shield lakes as alternative Archaean ocean analogues by studying microbial community dynamics in two such lakes in northwestern Ontario. Using 16S rRNA gene sequencing, we identified *Chlorobi* closely related to *C. ferrooxidans* as a dominant group in both lake anoxic zones over two summers. *Chlorobi* were accompanied by potential iron-reducing bacteria, sulfur-cycling bacteria, and methanotrophs, with shared dominant genera to those reported in another Archaean ocean analogue. Metagenome sequencing of anoxic zone samples confirmed that genes involved in sulfur cycling and methanotrophy were at high relative abundance to taxonomic marker genes. Detected *Chlorobi* were also found to have sulfur oxidation genes, raising the possibility for concurrent phototrophic iron- and sulfur-oxidation. No iron oxidation gene is known for this group. Overall, our findings highlight the consistency of microbial communities across boreal shield lakes and years despite reoxygenation and show metabolically unique communities potentially useful for extrapolation to early Earth ecosystems. The potential involvement of boreal shield lake anaerobic microbial communities in iron, sulfur, and methane cycling has implications for modern limnology and early Earth research. Boreal lake anoxia is expected to increase in prevalence and duration with climate change, making the contribution of detected communities to lake carbon balance, or iron fertilization of surface algal blooms, potentially important at global scales. Moreover, having millions of ferruginous lakes opens the possibility for inferring early Earth ecosystem controls by studying how modern microbial communities shift along environmental gradients. Boreal shield lakes present an unexpected and potentially transformative research platform to address questions of ancient and modern significance due to their robust anaerobic microbial consortia.

II PC 01

Inhibition and Structure of Peptidoglycan *O*-Acetyltransferase B from *Neisseria gonorrhoeae.* Ashley BROTT, University of Guelph, A.J. CLARKE[1], [1]University of Guelph

The peptidoglycan (PG) sacculus is unique to bacterial cell walls and holds a crucial role in maintaining cellular viability. Loss of integrity of this cellular armour, from actions of the host immune system or antibiotic treatment, is fatal. For years antibiotics such as beta-lactams, which target PG synthesis, have been used to combat bacterial infection. The flexible nature of both these systems and stem peptide composition allow for the emergence of bacterial resistance mechanisms which reek havoc in clinical settings, making the development of novel antibiotics a necessity. The PG sacculus is composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid (MurNAc), however unlike the stem peptide, the glycan backbone is conserved in all PG. This suggests that changes would not be trivial, leading us to hypothesize that novel antibiotics targeting the glycan backbone could provide a viable and longer sustaining antibacterial target. O-Acetylation of MurNAc is a major virulence factor as it renders the pathogen less susceptible to the host immune system leading to prolonged infection and increased pathogenicity. *O*-AcMurNAc has been identified in many pathogens including *Neisseria gonorrhoeae, Helicobacter pylori, Staphylococcus aureus,* and *Streptococcus pneumoniae*, all of which were identified in February 2017 by WHO as priority pathogens in need of research and development of new antibiotics. Peptidoglycan *O*-acetyltransferase (Pat)B is one of the enzymes responsible for this modification in Gram-negative pathogens. We hypothesize that the inhibition of PatB will lead to a decreased level of PG O-acetylation and increased sensitivity to lysozyme. An *in vitro* assay was used in a high throughput screen to monitor inhibition of PatB esterase activity were esculin and esculetin were identified as hits. Given the shared coumarin backbone of these two molecules, the coumarin family was chosen for further analysis. Half maximal inhibitory concentrations for esculin and esculetin were both found to be roughly 55 uM for PatB from *N. gonorrhoeae*. These compounds are also active against the Gram-positive O-acetylation system and when tested *in vivo* against *S. aureus* a minimum inhibitory concentration of 512 ug/ml was found for esculetin. The structure of PatB from *N. gonorrhoeae* was also determined to 1.25Å and to our knowledge this is the first structure to be determined for a

peptidoglycan *O*-acetyltransferase from any Gram-negative bacteria. This study identified the first inhibitors of PatB from *N. gonorrhoeae,* which can be used to study effects of inhibition and validate its potential as a novel drug target. The first structure of a PG *O*-acetyltransferase from Gram-negative bacterial pathogens, PatB, was solved giving structural insight into this poorly understood virulence system. This study paves the way for an increased understanding of the PG O-acetylation pathway, which has the potential to open new avenues for the development of antibiotics.

II PC 02

The transcriptional regulator SsrB acts as a molecular switch controlling *Salmonella* virulence gene expression. N. Y Elizabeth CHAU, McMaster University, D. T. MULDER[1], D PÉREZ-MORALES[2], M. M. BANDA[2], H SALGADO[2], I MARTÍNEZ-FLORES[2], J. A. IBARRA[2], B ILYAS[1], V. H. BUSTAMANTE[2], B. K. COOMBES[1], [1]McMaster University, [2]Universidad Nacional Autónoma de México

Bacteria reside in multiple independent niches and show a remarkable degree of plasticity in gene content and regulatory signaling pathways. In response to changing environments, bacteria tightly regulate the spatiotemporal expression of their gene products. This tight regulation is typically mediated by the interactions between transcription factors and sequence motifs upstream of gene coding regions. In a process called *cis*-regulatory evolution, the sequence motifs are subject to mutations that can lead to changes in the regulatory input and the transcriptional programs that are activated. However, the extent to which bacteria rewire their transcriptional regulatory circuitry in order to optimize virulence is unknown. *Salmonella enterica*is an enteric pathogen that causes acute gastroenteritis in human hosts. It has acquired two major gene islands called *Salmonella* pathogenicity island-1 and -2 (SPI-1 and SPI-2) each encoding a type III secretion system and effector proteins that are required for host-cell invasion and intracellular survival respectively. In this work, we investigated how the acquisition of the transcriptional regulator SsrB found in SPI-2 impacts the gene regulatory network in *Salmonella*. Using molecular and quantitative approaches, we show that the acquisition SsrB represses SPI-1 gene expression and *Salmonella* invasion of epithelial cells. We were able to demonstrate using electrophoretic mobility shift assays combined with transcriptional reporter fusions that SsrB directly binds to and represses the promoters of the master SPI-1 regulators, *hilD* and *hilA*, resulting in the repression of the downstream SPI-1 regulatory cascade. These data suggest that mutations occurring upstream of *hilD* and *hilA* have created novel SsrB binding sites, allowing a single protein regulator to activate intracellular virulence while simultaneously repressing genes no longer needed for an extracellular lifestyle. Our findings highlight how the re-wiring of gene regulatory inputs can fine-tune virulence gene expression in pathogenic bacteria and facilitate their adaptation to a host.

II PC 03

Antifungal Activity of the Human Gut Metabolome. Carlos GARCIA-RANGEL, Université de Laval, F TEBBJI [1], MC DAIGNEAULT[2], NN LIU[0], JR KO¨HLER[0], E ALLEN-VERCOE [2], A SELLAM[1], [1]Université de Laval, [2]University of Guelph

The human gut contains a variety of commensal microbes which are composed of diverse organisms that belong to all three domains of life with Eukaria primarily represented by fungi. The commensal/opportunistic yeast *Candida albicans* has been reported as the most common fungus in the gut of healthy humans. Recent study has shown that commensal microbiota play a critical role in the protection of the gut against colonization by other bacterial pathogens and pathobionts. However, so far, whether *C. albicans* overgrowth or pathogenicity are controlled by other fecal microbiota is not known. In this study, we showed that the secreted human gut metabolome (HGM) exerts an antifungal activity against different intestinal-resident yeasts including *C. albicans*, *C. tropicalis* and *C. parapsilosis.* The HGM inhibited the growth of both sensitive and drug-resistant strains of *C. albicans.* To uncover the mechanism of action of the HGM

associated with its antifungal property, a genome-wide genetic screen was undertaken and identified key components of the conserved TOR (Target Of Rapamycin) pathway as required for HGM tolerance. The HGM reduced the phosphorylation state of the known TOR effector, RPS6 confirming thus that the HGM inhibit *C. albicans* growth through TOR pathway. Given the central role of TOR pathway in modulating cell growth in response to nutrients cues, these data support a model where gut microbial cohabitants control *C. albicans* nutritional competitiveness through the modulation of TOR pathway activity. Furthermore, hyphal growth, a critical virulence trait of *C. albicans*, as well as the inducibility of hyphae-specific genes were significantly reduced. In accordance with the inhibitory effect on hyphae formation, we also showed that the HGM reduced significantly the damage inflicted to the human colon epithelial cells by *C. albicans*. To identify microbial specie(s) that produce the antifungal molecule(s), individual isolates form continuous-culture chemostat of fecal extracts of two healthy human donors were screened. Our data revealed that common butyrate-producing bacteria from the two donors exhibited an apparent antifungal activity. Together, these emphasize that the novel cross-kingdom interaction mediated by butyrate-producing bacteria contribute to the control of both the growth and virulence traits of *C. albicans* and provide a new paradigm where the commensal growth of this major human pathogen in the gut is dictated by the surrounding microbiota.

II PC 04

Human Milk Microbiota in the Canadian Healthy Infant Longitudinal Development (CHILD) Cohort.

Shirin MOOSSAVI, University of Manitoba, E. KHAFIPOUR[1], S. SEPEHRI[1], A. B. BECKER[1], P. J. MANDHANE[2], P. SUBBARAO[3], S. ?E. TURVEY[4], D. L. LEFEBVRE[5], M. R. SEARS[5], CHILD STUDY INVESTIGATORS[5], M. B. AZAD[1], [1]University of Manitoba, [2]University of Alberta, [3]University of Toronto, [4]University of British Columbia, [5]McMaster University

Background: Previous small-scale studies suggest that human milk contains a complex microbial community. However, milk microbiota composition has not been studied in a large cohort. **Methods**: We studied the milk microbiota of 395 lactating mothers in Canadian Healthy Infant Longitudinal Development (CHILD) study by sequencing the V4 region of the 16S rRNA gene on Illumina MiSeq. Each mother provided one milk sample at 4 months postpartum (median 16 weeks, interquartile range 14-19 weeks). We assessed α-diversity within samples (richness, diversity, and evenness indices) and β-diversity between samples (based on Bray-Curtis dissimilarity). The effect of lactation stage (time postpartum) on α-diversity was assessed by Pearson correlation and on β-diversity by analysis of similarity (anosim). Samples were grouped by hierarchical clustering with the ward sum of square algorithm. **Results:** Overall, 13 phyla and 170 genera were identified. Proteobacteria and Firmicutes were the most abundant phyla and were strongly negatively correlated ($r = -0.96$, $p < 0.001$). Average richness (observed single genus), diversity, and evenness at genus level were 114, 7.43, and 0.44, respectively. The most abundant genera were *Streptococcus* (17.1±17.4%), an unclassified *Oxalobacteraceae* (14.8±8.9%), and *Acinetobacter* (6.9±15.3%). However, some communities were dominated by a single taxa with a maximum abundances of up to ~90% for *Pseudomonas*, *Acinetobacter*, *Staphylococcus*, or *Streptococcus*. Three distinct groups emerged from hierarchical clustering. Proteobacteria-to-Firmicutes ratio ($p < 0.001$), number of observed genera ($p < 0.001$), and diversity ($p < 0.001$) were significantly different between clusters. Cluster 1 (n=90, least diverse) was dominated by *Acinetobacter* (23.6%), *Erwinia* (10.74%), *Pseudomonas* (14.25%), and *Staphylococcus* (10.46%); while cluster 2 (n=164) was dominated by *Streptococcus* (33.48%) and an unclassified Oxalobacteraceae (13.45%); and in clusters 3 (n=141, most diverse), an unclassified Oxalobacteraceae (23.7%) and an unclassified Comamonadaceae (9.54%) were most abundant. Within the range we evaluated, lactation stage was not significantly correlated with milk microbiota richness, diversity, evenness or overall β-diversity. **Conclusion:** Human milk contains a complex microbial community. Its potential contribution to

establishment of the infant gut microbiota, immunity, and health is the subject of ongoing investigation in the CHILD study.

II PC 05

Biofilm Stimulation in Response to Sub-Inhibitory Levels of Thiostrepton Reveals Unexpected Activity Against *Pseudomonas aeruginosa*. Michael RANIERI, McMaster University, N. SHARMA[1], U.T. NGUYEN[1], L.L. BURROWS[1], [1]McMaster University

Pseudomonas aeruginosa is a ubiquitous biofilm-forming gram-negative opportunistic pathogen that is responsible for many severe nosocomial infections. *P. aeruginosa* biofilms are tolerant of antibiotics because of their physiological heterogeneity and exclusion of antibiotics by the dense extracellular matrix. It has been observed that exposure of *P. aeruginosa* to antibiotics at levels below the minimum inhibitory concentration (MIC) induces an increase in biofilm formation. The presence of this biofilm response in *P. aeruginosa* and other organisms suggests that the stimulation of biofilm formation may constitute a general response to antibiotics. A screen for biofilm stimulating compounds was performed by our lab and identified the antibiotic thiostrepton as a biofilm stimulator. However, thiostrepton was not known to be an effective antibiotic against *P. aeruginosa*. Further work determined that the biofilm stimulatory activity was accompanied by growth inhibition in low iron culture conditions. From these results, we hypothesized that thiostrepton is acting in its canonical manner by targeting the ribosome and that it is gaining access to the cell via a siderophore uptake pathway. To test these hypotheses, we heterologously expressed a thiostrepton resistance protein, Tsr, in laboratory strains of *P. aeruginosa*. Tsr methylates ribosomal rRNA and abolishes thiostrepton activity. Strains expressing Tsr showed complete resistance to thiostrepton, indicating that thiostrepton was inhibiting the ribosome. To identify the import mechanism for thiostrepton, we screened select *P. aeruginosa* transposon mutants in iron-uptake related genes as well as a curated transposon mutant library for resistance to thiostrepton. This identified *tolQ* and *yidC* transposon mutants that were resistant to thiostrepton. Tol proteins, including *tolQ*, have been shown to be important for uptake of certain colicins and pyocins. This indicates that thiostrepton may use a similar mechanism to gain access to the cell. We also tested the thiostrepton susceptibility of 96 multi-drug resistant (MDR) *P. aeruginosa* clinical isolates from the Wright Clinical Collection. Our results indicated that 89 isolates had an IC_{50} of 5µM thiostrepton or less, which illustrates that thiostrepton or its derivatives may be useful for treating MDR infections. These results show that thiostrepton or other thiopeptides may prove useful as novel therapies to treat MDR *P. aeruginosa* infections. This work also highlights a potential entry mechanism that can be exploited by other drugs to make *P. aeruginosa* and potentially other gram-negative species more susceptible to antibiotics. Future work should aim to characterize and harness the biofilm response to sub-MIC antibiotics to screen for new drugs, as it presents a novel screening method for antibiotics with increased sensitivity over current assays.

MGCM PC 01

Investigating the regulatory roles of G-quadruplex structures in *Streptomyces*. Savannah COLAMECO, McMaster University, M. A. ELLIOT[1], [1]McMaster University

Streptomyces bacteria are best known for their capacity to produce an incredible range of natural products, including many compounds having antibiotic, anti-tumor, and anti-fungal activity. Despite this diversity of known natural products, genome mining of *Streptomyces* species has revealed many transcriptionally silent biosynthetic gene clusters encoding unknown products. This suggests that the biosynthetic potential of *Streptomyces* remains dramatically underexploited. There are many unknowns regarding gene regulation in *Streptomyces*, and a better understanding of this will undoubtedly assist in antibiotic discovery efforts. This work aims to fill some of these gaps by exploring the role of G-quadruplex (GQ) structures in gene regulation. GQ structures form in G-rich nucleic acid sequences, and serve important regulatory functions in

higher organisms. However, much less is known about the regulatory role of these structures in bacteria. Being extremely GC-rich organisms (>70%), *Streptomyces* are great candidates for studying GQs in bacteria. We are combining bioinformatic analyses of *Streptomyces* genomes, with experimental validation of GQ formation using reporter assays, *in vitro* experiments, and *in vivo* isolation of GQ structures. To date, we have identified ~2,500 GQ-forming sequences in each of three *Streptomyces* species, many of which are found in biosynthetic clusters. Through a series of transcriptional reporter constructs, we have shown that GQ-forming sequences affect gene expression *in vivo* and that the effect on gene expression depends on the distance of the GQ sequence from the promoter. While GQs near the promoter tend to cause a decrease in the expression of the downstream reporter, GQs further downstream from the promoter result in increased reporter expression. We hypothesize that GQs forming in the RNA may increase transcript stability by preventing RNase-mediated degradation. To test this, we are conducting *in vitro* RNA stability assays, and reporter assays in RNase deletion mutant backgrounds. We are also expanding our *in silico* analysis to specifically explore the effects of GQs in 5' and 3' untranslated regions (UTRs) to gauge the scope of this type of regulation in *Streptomyces*. This study is addressing fundamental questions about gene regulation in *Streptomyces* by exploring a completely novel mode of gene regulation in these organisms. We anticipate that our findings could be used to develop *Streptomyces*-specific genetic tools which will facilitate antibiotic discovery. Our findings on GQ-based gene regulation also have the potential for broad applicability in any bacterial species, as these structures are pervasive among bacteria.

MGCM PC 02

Genome-wide identification and characterization of small RNAs in *Rhodobacter capsulatus* and identification of small RNAs affected by loss of the response regulator CtrA. Marc GRÜLL, Memorial University of Newfoundland, L. LOURDES PEÑA-CASTILLO[1], M.E. MARTIN E. MULLIGAN[1], A.S. ANDREW S. LANG[1], [1]Memorial University of Newfoundland

Small non-coding RNAs (sRNAs) in bacteria are involved in the control of numerous cellular processes through various regulatory mechanisms, and in the past decade many studies have identified sRNAs in a multitude of bacterial species using RNA sequencing (RNA-seq). Here, we present the first genome-wide analysis of sRNA sequencing data in *Rhodobacter capsulatus*, a purple nonsulfur photosynthetic alphaproteobacterium. Using a recently developed bioinformatics approach, sRNA-Detect, we detected 422 putative sRNAs from *R. capsulatus* RNA-seq data. Based on their sequence similarity to sRNAs in RNA databases, 19 of the 422 putative sRNAs were assigned a predicted function, and the sequences of 105 additional putative sRNAs were conserved in at least one other bacterial species. We bioinformatically characterized all putative sRNAs and applied machine learning approaches to develop a quantitative model to calculate the probability of a nucleotide sequence to be an actual sRNA. The resulting logistic regression model was able to correctly classify 95.2% of nucleotide sequences in a validation set. We found that putative *cis*-targets for antisense and partially overlapping sRNAs are enriched with protein-coding genes involved in primary metabolic processes, photosynthesis, compound binding, and with genes forming part of macromolecular complexes. We performed differential expression analysis to compare the wild type strain to a mutant lacking the response regulator CtrA, an important regulator of gene expression in *R. capsulatus*, and identified 18 putative sRNAs with differing levels in the two strains. Finally, we validated the existence and expression patterns of four novel sRNAs by Northern blot analysis.

MGCM PC 03

PA3225 is a negative transcriptional regulator of the MexAB-OprM multidrug efflux pump in *Pseudomonas aeruginosa.* Clayton HALL, University of Ottawa, L ZHANG[1], T.-F. MAH[1], [1]University of Ottawa

Pseudomonas aeruginosa is a Gram-negative bacterium that can survive in a myriad of environments thanks, in part, to the large number of transcriptional regulators that it encodes in its genome. In humans, *P. aeruginosa* can act as an opportunistic bacterial pathogen that is an important cause of nosocomial infections. Unfortunately, infections caused by *P. aeruginosa* can be difficult to treat successfully due to the high level of intrinsic and adaptive antibiotic resistance that is displayed by this organism. A major determinant of antibiotic resistance in *P. aeruginosa* is the activity of multidrug efflux pumps, including MexAB-OprM. Given that transcriptional regulators are highly represented in the *P. aeruginosa* genome and since most of these regulators are uncharacterised, we hypothesised that some transcription factors that modulate intrinsic antibiotic resistance determinants, such as efflux pumps, may remain undiscovered in *P. aeruginosa*. In this work, we report on the characterisation of PA3225, a LysR-type transcriptional regulator. Unexpectedly, deletion of PA3225 led to small, but consistent, increases in the minimum inhibitory concentrations (MICs) of various antibiotics, including β-lactams, fluoroquinolones, chloramphenicol and tetracycline. The reduced antibiotic susceptibility of the ΔPA3225 mutant to a subset of these antibiotics was also demonstrated using antibiotic gradient plate assays. Intriguingly, however, the MICs of aminoglycosides were identical between the wild-type and ΔPA3225 mutant strains. Given that the ΔPA3225 strain was less susceptible to multiple structurally and functionally unrelated antibiotics, we reasoned that PA3225 might act as a repressor of one or more multidrug efflux pumps in *P. aeruginosa*. The expression of various multidrug efflux pumps, including MexAB-OprM, was determined in the ΔPA3225 mutant compared to wild-type by qPCR. Interestingly, in the ΔPA3225 mutant, the *mexAB-oprM* transcript was upregulated three-fold compared to wild-type. Gel shift assays were used to show that recombinant PA3225 was able to bind directly to the *mexAB-oprM* promoter, suggesting that PA3225 may act as a direct repressor of the *mexAB-oprM* operon. Levels of the MexB protein were also increased in the ΔPA3225 strain as determined by Western blot. In further support for the proposed role of PA3225 in negatively regulating *mexAB-oprM*, the ΔPA3225 mutant was less susceptible to antibiotics that are known substrates of the MexAB-OprM efflux pump. Deletion of PA3225 in a Δ*mexAB-oprM* background did not lead to a decrease in antibiotic susceptibility, further indicating that the reduced antibiotic susceptibility of the ΔPA3225 mutant was likely dependent on the activity of the MexAB-OprM multidrug efflux pump. Overall, these data support our conclusion that PA3225 acts as a transcriptional repressor of the genes encoding the MexAB-OprM efflux pump in *P. aeruginosa*.

MGCM PC 04

KpsC synthesizes a conserved β-linked poly-3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) linker in Gram-negative pathogen capsular polysaccharides. Doyle LIAM, University of Guelph, O.G. OVCHINNIKOVA[1], B.S. HUANG[2], M.S. KIMBER[1], T.L. LOWARY[2], C. WHITFIELD[1], [1]University of Guelph, [2]University of Alberta

Bacterial capsules are surface glycostructures important in host-pathogen interactions. Many Gram-negative pathogens such as extraintestinal pathogenic *Escherichia coli* and *Neisseria meningitidis* assemble CPS via a conserved pathway. A conserved feature of these CPS is a phospholipid attached, via a chain of β-linked poly-3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) units, to the variable CPS repeat-unit structure. The β-Kdo linker is synthesized by retaining β-Kdo glycosyltransferases (GTs), KpsS and KpsC. KpsS adds first and the dual domain KpsC extends it with multiple residues. KpsS and KpsC homologs are found in bacteria using the conserved pathway. KpsS and KpsC are members of the GT99 retaining β-Kdo GT enzyme family. The

crystal structure for a prototype GT99 enzyme contains features not found in other GTs. Additionally, β-Kdo is relatively rare outside this group of Gram-negative capsules. Thus, the enzymes synthesizing the β-Kdo linker are potential targets for small-molecule therapeutic development. The apparent absence of this capsule-assembly pathway in most commensal *E. coli* may lead to less collateral damage to the microbiome than broad-spectrum approaches. This research aims to determine the activity of each KpsC domain and confirm the structure of the conserved β-Kdo linker. These GT domains are hypothesized to possess β(2-4)-Kdo and β(2-7)-Kdo specificities accounting for the linkages in the natural product. However, the linkage order was unknown. The activity of each domain was examined independently using *in vitro* biochemical assays with recombinant KpsC proteins and synthetic acceptor molecules. Products were analyzed by MS and NMR spectroscopy to confirm their structures. Mutations to conserved binding pockets were made in each GT domain, resulting in proteins with single active domains; both domains are required to generate the native product. The N-terminal domain catalyzes β(2-4)-Kdo addition and was purified in active form. The C-terminal domain catalyzes β(2-7)-Kdo addition; however, activity of this domain was only detected in the wild-type protein or a mutant lacking N-terminal catalytic activity. The β-Kdo linker consists of an alternating sequence of these linkages. KpsC homologs were identified in a thermophilic Gram-negative bacterium, *Thermosulfurimonas dismutans*. Interestingly, *T. dismutans* produces two single domain homologs whose activities correlate to the N-terminal and C-terminal domains of *E. coli* KpsC, respectively. The purified enzymes show increased thermal stability compared to *E. coli* KpsC. These may present better candidates for high-throughput inhibitor screening and on-going structural characterization experiments. In summary, KpsC is a member of an enzyme family whose members catalyze β-Kdo addition to assemble glycostructures vital for pathogenic bacterial survival. Kdo is not found in humans, making KpsC a candidate drug target.

MGCM PC 05

Getting *Salmonella* Typhi's Vi antigen capsule to the cell surface. Sean LISTON, University of Guelph, O. G. OVCHINNIKOVA[1], C. WHITFIELD[1], [1]University of Guelph

Salmonella enterica serovar Typhi is a human pathogen that causes typhoid fever. The production of a polysaccharide capsule, also known as Vi antigen, is critical to the infection process. Vi antigen capsule assembly employs an **A**TP-**b**inding **c**assette (ABC) transporter. This strategy is conserved for Gram-negative bacterial **c**apsular **p**oly**s**accharides (CPS), with examples provided by extraintestinal pathogenic *Escherichia coli, Campylobacter jejuni, Kingella kingae*, *Haemophilus influenzae* and *Neisseria meningitidis*. These CPS are built on a glycolipid comprised of (lyso)phosphatidylglycerol attached to up to 10 residues of the sugar Kdo. CPS synthesis occurs in the cytosol and the completed molecule is then flipped across the inner membrane by the transporter. The nascent glycan transits the periplasm in a process requiring an inner membrane **p**olysaccharide **c**o-**p**olymerase (PCP) and **o**uter membrane **p**olysaccharide e**x**port (OPX) protein complex. The PCP and OPX proteins are proposed to form a contiguous channel from the transporter to the extracellular milieu. This work probes the model in which Vi antigen (i) is attached to the cell surface by an unusual terminal glycolipid, and (ii) transits the cell envelope via the PCP-OPX protein complex. Vi antigen-producers lack the ability to synthesize the (lyso)phosphatidylglycerol-Kdo_n acceptor. Instead, Vi antigen possesses a reducing terminal di-beta-hydroxy acyl-*N*-acetylhexosamine residue. We propose that these fatty acids arose from the putative acyltransferase, VexE, which is unique to Vi antigen biosynthesis. We found VexE directly acylated UDP-GlcNAc *in vitro*. Liquid chromatography coupled to mass spectrometry (MS) confirmed the reaction product was beta-hydroxymyristoyl-GlcNAc-1-phosphate. In our current model, the secondary acylation step is performed by another cytosolic acyltransferase and the lipid A secondary acyltransferases LpxL and LpxM are candidates for this activity. The Vi antigen biosynthesis locus in *Achromobacter denitrificans* encodes a Vi antigen depolymerase, VexL. Periplasmic polysaccharide-modifying proteins are not found in other ABC transporter-dependent CPS-assembly systems. We expressed

VexL in *S*. Typhi to ask whether Vi antigen was exposed during different stages of its assembly. Expression of cytosolic VexL did not affect Vi antigen production, suggesting that Vi antigen is assembled within a protected compartment or that synthesis is directly coupled to export. In contrast, expression of VexL in the periplasm degraded the Vi antigen, suggesting that CPS is not protected inside the proposed PCP and OPX protein channel during secretion. Understanding these conserved biosynthetic steps for CPS biosynthesis will allow for development of new antimicrobial targets/therapies.

MGCM PC 06

PilWXY1 contribute to type IV pilus-independent virulence of *Pseudomonas aeruginosa.* Victoria MARKO, McMaster University, S.L.N. KILMURY[1], L.T. MACNEIL[1], L.L. BURROWS[1], [1]McMaster University

The type IV pilus is a motility organelle found in a broad range of bacteria and archaea, including the opportunistic pathogen *Pseudomonas aeruginosa*. These flexible fibres are implicated in twitching motility, biofilm formation, and surface adhesion. The principle structural protein of the pilus fibre is the major pilin, PilA, while a set of low abundance proteins, called minor pilins, are proposed to constitute the pilus tip. The minor pilins, FimU-PilVWXE, along with the non-pilin protein PilY1, prime pilus assembly. PilVWXY1 feedback-inhibit transcription of the *fimU-pilVWXY1E* operon, which is positively regulated by the FimS/AlgR two-component system. In addition to its involvement in pilus assembly, PilY1 is an adhesin and putative mechanosensor that triggers virulence towards amoeba in response to surface attachment. PilWXY1 also promote c-di-GMP production via the diguanylate cyclase SadC, leading to increased biofilm formation and repression of swarming motility. In this study, we aimed to uncover the mechanism for PilWXY1-mediated virulence. We hypothesized that loss of the minor pilins and PilY1 would relieve the feedback inhibition on FimS/AlgR, resulting in the repression of acute virulence factors in the AlgR regulon.
A *Caenorhabditis elegans* slow killing assay was used to screen for differences in virulence between PA14 and PAO1 *pilA*, *fimU*, *pilV*, *pilW*, *pilX*, *pilY1*, and *pilE* mutants. *pilW*, *pilX*, and *pilY1* mutants had reduced virulence relative to wild type and a *pilA* mutant, implying a role in pathogenicity independent of type IV pilus assembly. Deletion or overexpression of *sadC* had no impact on virulence, suggesting the PilWXY1/SadC pathway is not important for pathogenesis in *C. elegans*. In contrast to published data, the PA14 *pilW*, *pilX*, and *pilY1* mutants had swarming motility defects, indicative of higher c-di-GMP levels. The reduced swarming and virulence of the *pilW*, *pilX*, and *pilY1* mutants was dependent on FimS/AlgR, and the overexpression or hyperactivation of AlgR led to reduced virulence towards *C. elegans*. These data support a model where the loss of PilWXY1 promotes *fimS-algR* transcription, leading to overall higher levels of phosphorylated AlgR, and therefore repression of acute virulence genes. Hyperactivation of AlgR is also thought to promote the expression of genes associated with chronic infection; this possibility should be considered if PilWXY1 are to be targeted with anti-virulence drugs.

MGCM PC 07

Endoplasmic Reticulum Stress Modulates Herpes Simplex Virus Type 1 Gene Expression and Replication. Mackenzie THORNBURY, Dalhousie University, E.P. PRINGLE[1], C. MCCORMICK[1], [1]Dalhousie University

Herpesviruses are ubiquitous human pathogens and obligate intracellular parasites that must infect a host cell to replicate and spread. Viral infection burdens the host protein synthesis and secretory apparatus, causing endoplasmic reticulum (ER) stress that activates the PKR-related ER kinase (PERK). PERK inhibits translation by phosphorylation of an important regulator, eukaryotic initiation factor 2 alpha (eIF2α). Phosphorylation of eIF2α selectively prevents translation initiation at canonical AUG start codons, which would be expected to limit synthesis of viral proteins. The mechanisms that permit ongoing viral protein synthesis despite eIF2α phosphorylation remain poorly understood. Initiation of protein synthesis from non-AUG start codons, such as CUG, is independent of eIF2α, and utilizes a distinct set of translation initiation factors. Comparative bioinformatic analysis of herpesvirus genomes reveals abundant in-frame non-AUG start codons that could permit translation of viral proteins despite eIF2α phosphorylation. Here we show that pharmacologically increasing ER stress and eIF2α phosphorylation during HSV-1 infection does not inhibit virus replication or spread. Furthermore, we can detect the emergence of new, stress specific HSV-1 protein species. Our findings suggest herpesviruses may use non-canonical translation initiation mechanisms to allow the accumulation of viral proteins during times of stress.

MGCM PC 08

A multifunctional protein acts as a polymerase, terminator and chain-length regulator in the biosynthesis of lipopolysaccharide O-antigen in *Klebsiella pneumoniae* O12. Danielle WILLIAMS, University of Guelph, DM WILLIAMS[1], OG OVCHINNIKOVA[1], A KOIZUMI[1], MS KIMBER[1], IL MAINPRIZE[1], TL LOWARY[2], CW WHITFIELD[1], [1]University of Guelph, [2]University of Alberta

Lipopolysaccharide (LPS) is a cell surface glycolipid which is found in the outer membrane of Gram-negative bacteria. It is made up of three components the lipid A, core oligosaccharide, and O-antigenic polysaccharide (OPS). LPS synthesis occurs in two separate assembly and export processes with the lipid A and core oligosaccharide being synthesized in a sequential pathway and OPS being synthesized separately. The two parts are then ligated at the periplasmic face of the inner membrane. OPS biosynthesis can occur via one of three possible pathways all of which are comprised of inner membrane enzymatic complexes. This study focuses on the ABC transporter-dependent pathway. In a prototype provided by the *Escherichia coli* O9a antigen the characteristic (preferred) range of glycan chain lengths is established by the action of two proteins; a polymerase containing two glycosyltransferase catalytic sites with different activities and a chain-terminating enzyme. The polymerase interacts with the membrane bound terminator but the active sites for chain extension and chain termination are separated by a rigid coiled-coil structure that acts as a molecular ruler to determine glycan chain length. Here, we describe a related system found in *Klebsiella pneumoniae* and *Raoultella terrigena* , where the polymerase and terminator activities are provided by separate domains within a single protein, WbbB. The two glycosyltransferase catalytic sites found in the polymerase belong to the newly discovered GT102 and GT103 families and are involved in generating the [4)-αRha-(1-3)-βGlcNAc-(1] repeat-unit of the OPS. The specificities of these catalytic sites were investigated *in vitro,* using purified proteins, the activated precursors (dTDP-Rha and UDP-GlcNAc) and synthetic acceptors terminating in either α1,3-linked Rha or β1,4-linked GlcNAc. The reaction products were verified by MS and NMR methods. Both active sites are required to make authentic O12 polysaccharide antigen. The GT102 domain possesses the rhamnosyltransferase activity and is active as a separate polypeptide. The GT103 domain only retains activity when in the presence of the GT102 domain, therefore it was expressed alongside a catalytically inactive GT102 and was confirmed to act as the N-

acetylglucosaminyltransferase. The activity of the terminator, a β-Kdo transferase, has been established previously by our group. This activity is separated from the polymerase by a putative coiled-coil structure. The chain length of the O12 polysaccharide produced *in vivo* was responsive to systematic changes in the length of the coiled-coil confirming its role as a molecular ruler. The inclusion of all of these key activities within a single protein, WbbB, provides new insight into the assembly of bacterial polysaccharides.