## Student Symposium Competition Abstracts

Note:

**AEM:** Applied Environmental Microbiology

**II:** Infection and Immunity | **MGCM:** Molecular Genetic Cellular Microbiology

### STUDENT SYMPOSIUM I
### Wednesday, June 21st, 2017
### 3:00-4:30 PM

AEM SSC 01

**Neonicotinoid-induced pathogen susceptibility is mitigated by *Lactobacillus plantarum* immune stimulation in a *Drosophila melanogaster* model**

Brendan DAISLEY, University of Western Ontario, MT TRINDER[1], TWM MCDOWELL[2], HW WELLE[3], JSD DUBE[1], SNA ALI[1], HSL LEONG[1], MWS SUMARAH[2], GR REID[1], [1]University of Western Ontario, [2]Agriculture and Agri-Food Canada, [3]Vrije Universiteit Amsterdam, HV Amsterdam, Netherlands

Pesticides are used extensively in food production to maximize crop yields. However, neonicotinoid insecticides exert unintentional toxicity to honey bees (*Apis mellifera*) that is partially associated with massive population declines referred to as colony collapse disorder. We hypothesized that imidacloprid (common neonicotinoid; IMI) exposure would make *Drosophila melanogaster* (an insect model for the honey bee) more susceptible to bacterial pathogens, heat stress, and intestinal dysbiosis. Our results suggested that the immune deficiency (Imd) pathway is necessary for *D. melanogaster* pro-survival in response to IMI toxicity. IMI exposure induced alterations in the host-microbiota as noted by increased indigenous *Acetobacter* and *Lactobacillus* spp. Furthermore, sub-lethal exposure toIMI resulted in decreased *D. melanogaster*survival when simultaneously exposed to bacterial infection and heat stress (37°C). This coincided with exacerbated increases in *TotA* and *Dpt* (Imd downstream pro-survival and antimicrobial genes, respectively) expression compared to controls. Supplementation of IMI-exposed *D. melanogaster*with *Lactobacillus plantarum* ATCC 14917 mitigated survival deficits following *Serratia marcescens* (bacterial pathogen) septic infection. These findings support the insidious toxicity of neonicotinoid pesticides and potential for probiotic lactobacilli to reduce IMI-induced susceptibility to infection.

II SSC 01

**Spontaneous preterm birth and the vaginal microbiome**

Aline FREITAS, University of Saskatchewan, A BOCKING[1], DM MONEY[2], JE HILL[3], VOGUE STUDY-TEAM[2], [1]University of Toronto, [2]University of British Columbia, [3]University of Saskatchewan

The bacterial community (microbiome) present in the female lower genital tract plays an important role in maternal and neonatal health. Imbalances in this microbiome have been associated with negative reproductive outcomes, such as spontaneous preterm birth (sPTB), but the mechanisms underlying the association between a disturbed microbiome and sPTB remain poorly understood. Intrauterine infection ascending from the vagina may be an important contributor to the onset of labour. Our objective was to characterize the vaginal microbiome of pregnant women who had sPTB (n=46) and compare to those of

pregnant women who had a term delivery (n=170). Vaginal swabs and demographic/lifestyle questionnaires were collected from women at 11-16 weeks of gestational age (GA).Total nucleic acid was extracted and microbiome profiles were created by PCR amplification and pyrosequencing of the *cpn*60 universal target region. Samples were screened for the presence of Mollicutes (*Mycoplasma* or *Ureaplasma*) using genus-specific PCR. There were no significant differences in age, BMI, ethnicity, smoking status, or consumption of alcohol and probiotics between women who delivered at term and preterm ($p>0.05$).Average GA at delivery were $39^{+3}$ and $34^{+2}$ weeks$^{+days}$ for women in the term and preterm groups, respectively. Most women (67%) who had sPTB were considered late preterm (34-$36^{+6}$ GA). Microbiomes clustered into seven Community State Types (CST): I (*Lactobacillus crispatus* dominated), II (*Lactobacillus gasseri* dominated), III (*Lactobacillus iners* dominated), IVA (*Gardnerella vaginalis* subgroup B or mix of species), IVC (*G. vaginalis* subgroup A dominated), IVD (*G. vaginalis* subgroup C dominated) and V (*Lactobacillus jensenii* dominated). The microbiomes of women who had sPTB had higher richness and diversity, and Mollicutes prevalence when compared to those of women who delivered at term ($p<0.05$). A few rare species were also more prevalent in women who had sPTB. Women in the preterm group were more likely to have experienced sPTB or miscarriage in a previous pregnancy ($p<0.05$).The groups did not cluster according to CST, likely because CST assignment is driven in most cases by the dominance of one particular species, overwhelming the contributions of more rare taxa. In conclusion, we did not identify a specific microbial community structure that predicts sPTB. Differences in microbiome richness, diversity and Mollicutes prevalence, however, were observed between groups. Although a causal relationship remains to be determined, our results confirm previous reports of an association between Mollicutes and sPTB, and further suggest that rare bacteria may be important in the pathogenesis of some cases. Future studies focusing on characterization of low abundance species and the metabolite production of themore diverse microbiome associated with sPTB might further elucidate factors leading to sPTBand identifywomen at risk early in pregnancy.

MGCM SSC 02

**Characterization of an anti-CRISPR that can convert a type I-E CRISPR-Cas complex into a transcriptional activator**

Marios MEJDANI, University of Toronto, MM MEJDANI[1], AP PAWLUK[1], KLM MAXWELL[1], ARD DAVIDSON[1], [1]University of Toronto, Toronto ON

Anti-CRISPRs are protein inhibitors of CRISPR-Cas systems and are produced by phages to evade CRISPR-Cas-mediated destruction. We have discovered more than 15 families of anti-CRISPRs that inhibit three different CRISPR-Cas systems. These anti-CRISPRs function through a variety of mechanisms and we are characterizing these mechanisms with the aims of gaining further insight into CRISPR-Cas function, and developing new anti-CRISPR-based biotechnological tools. In this study, we focused on AcrE2, an inhibitor of the Type I-E CRISPR-Cas system in *Pseudomonas aeruginosa*. We determined the structure of this anti-CRISPR using NMR spectroscopy. Using the structure coupled with site-directed mutagenesis, we identified a small cluster of residues that are required for function. When we targeted the CRISPR-Cas system to a transcriptional promoter in the presence of AcrE2, we observed transcriptional repression, implying that the CRISPR-Cas complex could still bind DNA and thus blocked RNA polymerase from accessing the promoter. Surprisingly, when the CRISPR-Cas complex was targeted to a different region of the same promoter the presence of AcrE2 resulted in transcriptional activation. Importantly, targeting the CRISPR-Cas complex to the same promoter region in the absence of Cas3, the nuclease component of this system, resulted in transcriptional repression. These results imply that AcrE2 interacts with the DNA-bound CRISPR-Cas complex to change its conformation and cause increased transcription. Moreover, AcrE2 must also block the action of Cas3, likely preventing it from binding the CRISPR-Cas:DNA complex. These data show that AcrE2 is acting through a unique mechanism compared to other characterized anti-CRISPRs, and suggest that anti-CRISPRs could play roles as modulators of CRISPR-Cas function, not just inhibitors.

AEM SSC 02

**Antimicrobial resistance gene surveillance in the receiving waters of an upgraded wastewater treatment plant**

Claire FREEMAN, University of Regina, C.K. YOST[1], M.L. SCRIVER[1], [1]University of Regina, Regina SK

Antimicrobial resistance genes (ARGs) have become a recognized environmental contaminant. Although they can be detected in pristine environments with minimal anthropogenic influence, their measured abundance is elevated in managed environments, such as feedlots and wastewater treatment plants (WWTPs). WWTPs are of particular importance because they receive influent from hospitals and all municipal and commercial locations within the city; because of this, WWTPs represent a critical point where patterns in ARGs can be monitored prior to their release into the environment. The WWTP in Regina, Saskatchewan, Canada was recently upgraded to a bioreactor system for biological nutrient removal, including nitrogen. This upgrade took place in 2016 to accommodate recent federal restrictions on wastewater effluent, and thus represents an opportunity to investigate how new treatment processes will impact the release of antimicrobial resistance genes and bacteria into the environment. Sampling took place within the Wascana Creek Watershed, in the area immediately surrounding the Regina Municipal WWTP in southern Saskatchewan, Canada. Wascana creek receives the tertiary treated wastewater effluent; creek volume is low in comparison to the effluent-receiving rivers of other major Canadian. Thus, there is relatively little dilution power within the effluent system and therefore provides an interesting system to study the release of antibiotic resistance genes. Surface water was collected from Wascana Creek at three sites upstream of the plant and four sites downstream of the plant, in addition to final effluent (FE) directly from the plant. Between 150 and 200 ml of each sample was passed through a 0.45 um filter, which was retained and used for a total genomic DNA isolation. Quantitative PCR was used to measure the abundance of ten genes, including eight ARGs, the *intI1* gene, and the 16s rRNA gene. Six out of the nine genes surveyed were found in higher quantities immediately downstream of the plant, including *ermB, sul1*, *intI1*, *blaCTX-M*, *qnrS*, and *tetO*, consistent with the genes with the highest abundance in the FE. Most ARGs were present at consistent levels at all three upstream sites. With the exception of *blaTEM*, ARG abundance measured from the furthest downstream site had reductions in ARG abundance ranging from 12% to 88% when compared with the site closest to the effluent release point. Similar to the results of other studies, the Regina Municipal WWTP is releasing treated effluent that has high levels of ARGs relative to the surrounding environment. The plant made an impact on the abundance of most genes, with the noticeable exception of *vanA*and *mecA*, which were in low abundance upstream and downstream of the plant. These findings are consistent with the increasing pool of evidence suggesting that human activity greatly affects the abundances of ARGs in the environment.

II SSC 03

**Investigating the role of antibiotics and adherent-invasive *E. coli* in the pathogenesis of Crohn's disease**

Alexander OBERC, McMaster University, A. FIEBIG-COMYN[1], B.K. COOMBES[1], [1]McMaster University, London ON

Crohn's disease (CD) is an inflammatory bowel disease which causes a wide range of debilitating symptoms and is affecting an increasing number of Canadians. CD is a treatable but currently incurable condition that is associated with substantial costs on our healthcare system. CD is believed to have a complex etiology consisting of microbial, genetic, environmental, and immunological factors. Microbial factors in particular have been thought to play a central role in causing intestinal inflammation and dysbiosis of the intestinal microbiota has been found in CD patients. In addition, CD is also associated with increased abundance of an unusual phenotypic group of *Escherichia coli* known as adherent-invasive *E. coli* (AIEC) in many patients.

AIEC are characterized by their ability to adhere and invade various cell types, to stimulate the production of inflammatory cytokines. However, since AIEC are also found in healthy patients it is likely that additional CD risk factors are required for the development of CD. Multiple epidemiological studies have linked antibiotics with subsequent CD diagnosis. Antibiotics have been found to exacerbate dysbiosis in CD patients and to favour the growth of *E. coli*. Antibiotic resistance has also been found to be widespread in AIEC isolates. The impact of antibiotics on AIEC infection has not been well studied *in vivo*. Our lab has found that chronic colonisation of conventional mice with AIEC leads to intestinal inflammation and fibrosis. Our objective was to use this mouse model to investigate the impact of antibiotics on AIEC colonisation, pathology, and immune responses. Mice were treated with various antibiotics in drinking water or by oral gavage. These mice were infected with various doses of AIEC either before or after antibiotic treatment. We found that certain antibiotics administered prior to infection greatly reduced the infectious dose of bacteria required and led to greater bacterial burden. Administering antibiotics post AIEC infection similarly showed an expansion of AIEC in the feces and tissues and penetration of AIEC into underlying tissues. We are continuing to investigate how antibiotics alter AIEC colonisation by studying the metabolic and immune consequences of antibiotic treatment. These results strengthen our understanding of AIEC pathogenesis and of the impact antibiotics have on the intestine. Furthermore, these results provide greater insight into the complex relationship between microbial and environmental factors in the development of Crohn's disease

MGCM SSC 03

**A transcriptional regulatory system governs anti-CRISPR expression and phage viability**

Sabrina STANLEY, University of Toronto, A BORGES[1], J BONDY-DENOMY[1], AR DAVIDSON[2], [1]University of California, San Francisco, [2]University of Toronto, Toronto ON

Phage infection poses a major threat to bacterial survival. To protect themselves, bacteria have evolved numerous defence mechanisms including the widespread CRISPR-Cas systems, which provides sequence-specific protection against phage invasion. In turn, phages have evolved inhibitors of CRISPR systems. Our lab has discovered nine distinct families of "anti-CRISPRs" in phages of *Pseudomonas aeruginosa* that are all associated with a highly-conserved helix-turn-helix containing protein called anti-CRISPR associated 1 (Aca1). Here, we investigate the function of Aca1 and provide insight into its conservation. Through a series of *in vitro* studies and transcriptional reporter assays, we have found that Aca1 binds to a strong, constitutive anti-CRISPR associated promoter and that Aca1 represses its activity. We also determined that this promoter is critical for anti-CRISPR expression during infection. By mutagenesis, we determined that the repressor activity of Aca1 is essential as mutant phages are not viable, explaining its conservation. We show that the loss of phage viability is caused by uncontrolled expression from the anti-CRISPR promoter. Taken together, these data suggest that anti-CRISPRs have their own transcriptional regulatory system that ensures sufficient anti-CRISPR expression at the onset of infection and subsequent downregulation once the anti-CRISPR is no longer needed to ensure phage survival.

**STUDENT SYMPOSIUM II**
**Thursday, June 22nd, 2017**
**3:00 - 4:30 PM**

AEM SSC 03

**Characterization of Pap-Pit: a broadly distributed bipartite phosphate transport system in Bacteria and Archaea**

Daniel HSIEH, McMaster University, GC DICENZO[1], R ZAHEER[1], TM FINAN[1], [1]McMaster University, London ON

Phosphorus is an essential element to life and is a major component of vital biomolecules in nucleic acids, membrane phospholipids, and signal transduction. Microorganisms primarily acquire phosphorus in the form of inorganic phosphate ($PO_4^{-3}$ or Pi) through expression of a suite of phosphate scavenging or phosphate uptake systems in response to environmental phosphate. One such system is the Pit family of single protein Pi uptake systems found in all kingdoms. The canonical bacterial Pit protein is the *Escherichia coli* PitA protein, a 499 aa low-affinity, high-velocity transporter of Pi. Previously, the *pit* gene of the soil bacterium *Sinorhizobium meliloti*, was found to encode a 334 aa low-affinity Pi uptake system that is repressed during phosphate-limiting conditions. However, the *S. meliloti pit* gene is found in an operon and overlaps the coding sequence of a protein of unknown function, which was denoted as *pap* for *pit-accessory protein.* In this work, we demonstrate that Pap is an accessory protein required for phosphate uptake via the Pit protein.Using a conditional Pi-transport deficient mutant strain of *S. meliloti*, we studied the effects of *pap* or *pit* mutations on Pit mediated Pi uptake. Growth experiments were conducted in minimal media with Pi as the sole source of phosphorus, where growth was indicative of Pit mediated Pi transport. *S. meliloti* with either *pap* or *pit* null mutations were unable to grow, suggesting both Pap and Pit are required for Pit mediated Pi uptake. A total of 2180 bacterial and archaeal genomes were searched for Pap-Pit orthologs were using Pfam protein motifs of Pit (PHO4) and Pap (PhoU_div). Approximately 85% of *pap* orthologs co-occurred as an operon, and *pap-pit* operons were found in ~ 40% of bacterial and archaeal genomes. This suggested that the Pap-Pit system plays a crucial role in Pi uptake in many microorganisms. Thus, Pap-Pit represent a two-protein subtype of Pit family protein found exclusively in prokaryotes.The molecular mechanism of this modulating function via Pap-like proteins has not been elucidated and is the focus of current studies.

II SSC 04

**Comparative analysis of antimicrobial susceptibility and biofilm formation in *Escherichia coli* expressing laterally transmitted quaternary ammonium compound efflux pumps**

Carmine SLIPSKI, University of Manitoba, T JAMIESON[1], K BELL[1], GG ZHANEL[1], DC BAY[1], [1]University of Manitoba, Manitoba AB

Antimicrobial resistance genetic surveillance of multidrug-resistant proteobacterial isolates from hospitals, veterinary, and food handling facilities frequently detect the presence of quaternary ammonium compound efflux pump genes (*qac)*. Qac proteins are members of the small multidrug resistance (SMR) transporter family and confer resistance to a wide range of cationic antiseptics, disinfectants, and toxic dyes driven by proton motive force. *qac* genes are conserved in integrons of multidrug-resistant plasmids and frequently co-associate with other multidrug-resistant enterobacterial isolates such as extended-spectrum beta-lactamase (ESBL). Our aim is to compare multidrug-resistance phenotypes conferred by frequently identified representative *qac* members based on surveys of sequenced Gram-negative plasmids. Bioinformatic analysis of Qac sequences from over 1000 sequenced plasmids was performed to determine Qac phylogenetic relatedness and distributions among clinical, veterinary, food and environmental isolates. Phylogenetic

surveys of Gram-negative bacterial plasmids yielded four distinct clades, *qacE*/*Edelta1*, *qacF*, *qacG/H* and *sugE(YP)*, where *qacEdelta1* was most frequently detected and *qacG* the least frequently detected among plasmids surveyed. Based on this analysis, five representative *qac* members were cloned and selected for further analysis and compared to archetypical SMR family members *emrE* and *sugE* (NP). Antimicrobial susceptibility testing of *qac* members was determined using *Escherichia coli* K12 strains BW25113, KAM32, KAM42 to a collection of cationic antimicrobials: 9 antiseptics (quaternary ammonium compounds) and 2 antibiotics (tobramycin and erythromycin). The results supported the cladistic segregation of *qac* and *sugE* members and demonstrated that each *qac* member exhibited varying patterns of drug polyspecificity based on minimal inhibitory concentration (MIC) values. The biofilm forming potential of *qac* transformed strains indicated that only qac transformed *E.coli* KAM32 strains showed significant increases in biomass. These findings provide new antimicrobial resistance phenotypes for relevant antimicrobials and clarify the phylogenetic relatedness of diverse *qac* members. These findings are vital to ongoing genetic surveillance testing of multidrug-resistant isolates.

MGCM SSC 01

**A novel mode of bacterial development is triggered by interkingdom interactions and airborne signals**

Stephanie JONES, McMaster University, LKH HO[1], CAR REES[0], JEH HILL[0], JRN NODWELL[1], MAE ELLIOT[2], [1]University of Toronto,Toronto ON [2]McMaster University London ON

*Streptomyces* are ubiquitous soil bacteria, best known for their ability to produce an extraordinary range of medicinally useful specialized metabolites and for their complex life cycle. *Streptomyces* share their soil environment with multitudes of other microbes, and the consequences of microbial interactions on *Streptomyces* behaviour have not been thoroughly examined. It has long been thought that the *Streptomyces* life cycle encompasses three developmental stages: vegetative hyphae, aerial hyphae, and spores. In this work, we demonstrate interactions with yeast induce a novel form of *Streptomyces* development termed 'explorers'. These explorer cells grow as non-branching vegetative hyphae, and can rapidly transverse biotic and abiotic surfaces. Exploration is not confined to one *Streptomyces* species: 19/200 tested wild *Streptomyces* strains exhibited exploratory growth beside yeast. We found yeast triggers exploration indirectly by consuming glucose from the medium, relieving repression of exploration. Explorers raise the pH of the medium from 7.0 to 9.5 using an airborne volatile organic compound (VOC). Remarkably, we found *Streptomyces* explorers can induce exploration in physically separated streptomycetes using this alkaline VOC. Using gas chromatography/mass spectrometry and *ex vivo* assays, we determined that the alkaline VOC is trimethylamine (TMA). Additionally, we found TMA can inhibit the growth of other non-streptomycete soil bacteria, indicating this VOC can act as both a *Streptomyces* communication signal and a competitive tool. This work introduces a new mode of *Streptomyces* development triggered by interkingdom interactions and propagated by a VOC. These results reveal simple metabolic cues – such as glucose depletion or a rise in pH – can trigger dramatic and unexpected developmental responses. Furthermore, this work provides evidence that VOCs can act as both airborne communication signals and as competitive tools capable of eliciting changes in the behaviour and survival of neighboring microorganisms.

MGCM SSC 04

**Structure and molecular basis of catalysis of the peptidoglycan *O*-acetyltransferase catalytic domain**

David SYCHANTHA, University of Guelph, P.L. HOWELL[1], A.J. CLARKE[2], [1]University of Toronto, [2]University of Guelph

Lysozyme resistance caused by peptidoglycan (PG) O-acetylation contributes to successful colonization and persistence of *Staphylococcus aureus* and *Streptococcus pneumoniae*, both of which are commonly resistant to antibiotics. Strains lacking the enzyme OatA, which confers this modification, are less virulent and often more sensitive to β-lactam antibiotic treatment, suggesting its inhibition has potential for reducing the burden of these pathogens. To establish a biochemical basis for its function as means to guide future drug discovery, we reconstituted the activity of the catalytic domains of OatA from *S. aureus* and *S. pneumoniae in vitro*. Additionally, we determined the crystal structure of this domain from *S. pneumonia* and determined the details of its catalytic mechanism. Using chemo-enzymatically prepared undecaprenyl-linked PG chains, we distinguished distinct substrate specificities for the two enzymes, finding that stem-peptide length is profoundly important for *in vitro* catalysis. The structure of the OatA catalytic domain revealed an atypical α/β hydrolase fold. Using the structure as a guide, the active site residues were systematically replaced with alanine to assign catalytic or substrate binding roles. From this information a model is proposed that invokes the participation a Ser-His-Asp catalytic triad to O-acetylate peptidoglycan through a covalent acetyl-enzyme intermediate. Taken together, the data presented herein identify and characterize the role of the extracytoplasmic domain of OatA in the O-acetylation of nascent lipid-linked PG

AEM SSC 04

**Forests fueling lake sediments: exploring microbial decomposers responsible for greenhouse gas production**

Kurt M. YAKIMOVICH, Laurentian University, C. ORLAND[1], E.J. SZKOKAN-EMILSON[1], A.C. CARSON[2], J.T. TANENTZAP[1], N. BASILIKO[2], N.C.S. MYKYTCZUK[2], [1]Cambridge University, [2]Laurentian University, Sudbury ON

The fate of C that is transformed by lake sediment microbial communities can either lead to sequestration in sediments or mineralization to $CH_4$ and/or $CO_2$. A major C source for lake sediment microbial communities is terrestrial plant litter inputs. As terrestrial organic matter (TOM) is often more recalcitrant than internal lake sources, much of the resident C in lake sediments is terrestrial in origin. Therefore, here we explore how terrestrial litter inputs effect sediment microbial communities and C mineralization rates, in both an *in-vitro* anaerobic lab incubation and an *in-situ* mesocosm experiment. The *in-vitro* experiment used three types of plant litters, both a deciduous and coniferous tree litter mix typical of boreal catchments and cattails (*Typha latifolia*). Examination of the bacterial, fungal and methanogen communities via amplicon sequencing and qPCR revealed that OM type selected for unique microbial community compositions. The primary biochemical factor driving community composition in all three microbial communities was polyphenols. Polyphenol content of the litter selected for different dominant bacterial fermenters that varied among litter types. Polyphenols negatively affected methanogen communities, with relatively lower methanogen abundance and activity found in the deciduous amended sediments. Fungal community composition was largely defined by litter type, however higher overall abundance corresponded with higher polyphenol levels. The increase in fungal abundance was attributed to increases in the relative abundance of *Phlebia spp.*, a genus containing white-rot fungi known for producing polyphenol oxidases. Following up these results, a field based experiment was deployed in three lakes around Sudbury, ON. Mesocosms contained artificially constructed sediments using inorganic material mixed with a deciduous and coniferous tree litter mix. Sediments were constructed to provide both a concentration gradient of total OM, and

different ratios of deciduous:coniferous litter. During the first three months of decomposition, mesocosms were sampled to assess pore water chemistry and obtain sediment samples for amplicon sequencing of the methanogen community. Differences were observed in the methanogen community composition between all three lakes, and between litter amended mesocosms and controls. All mesocosms were primarily dominated by the same two methanogens, however as decomposition progressed the relative abundance of a specialized methanol reducer increased. The specialist methanogen was responsible for increases in $CH_4$ production and was found in all three lakes, and was most abundant in the lake whose mesocosms had the greatest photoexposure. Taken together this work reveals how differing source of OM can influence microbial community composition and activity based on different biochemical characteristics. Additionally, the field experiments show how physicochemical factors can influence methanogen communities. Understanding microbial dynamics in lake sediments reveals how lakes are intertwined in both local and global C-cycling, connecting forests and lakes to the atmosphere.

II SSC 02

**Regulatory evolution drives immune evasion by *Salmonella* Typhimurium**

Bushra ILYAS, McMaster University, DT MULDER[1], BK COOMBES[1], [1]McMaster University

Bacterial pathogens encounter a wide variety of host niches which require specific adaptations in gene expression. The bacterial pathogen *Salmonella* Typhimurium tightly regulates gene expression to subvert the immune system and to activate its intracellular virulence program. SsrA-SsrB is a horizontally-acquired two component system which detects a variety of environmental stimuli and has been identified as a master regulator of this intracellular lifestyle. Research in our lab has shown that mutations in *cis*-regulatory genetic elements have led to these genes being regulated by SsrB, and that these genes are important for *Salmonella* virulence. To identify novel pathways under the control of SsrB, we conducted comparative RNA-sequencing of *S.* Typhimurium and the closely related, SsrB-naive species *Salmonella bongori,* in the presence of SsrB under infection relevant conditions. These data identified numerous functional groups of genes that are differentially regulated in the SsrB-adapted *S.* Typhimurium, as compared to *S. bongori,* thus identifying novel infection relevant genetic regulation. In particular, we have identified a strong repression of genes involved in flagellar-based swimming motility. Here we have confirmed that swimming motility is differentially regulated in *S.* Typhimurium expressing constitutively active SsrB compared to a knockout strain, and that this difference does not exist when SsrB is introduced into *S. bongori*. I have also found that immune activator flagellin (FliC) is differentially regulated by SsrB using transcriptional reporter data and *in vitro* secretion assays. Future work will focus on demonstrating that this regulation is important for virulence by exploring the ability of these strains to activate the flagellin-sensing inflammasome complex in macrophages, which has been shown to be important in limiting *Salmonella* infections. These results will further our understanding of regulatory evolution and pathogenesis of *S.* Typhimurium. Furthermore, these results can be used as a model understanding how other organisms evolve in response to horizontally-acquired transcriptional regulators.

# POSTDOCTORAL RESEARCH SYMPOSIUM
## Friday, June 23rd, 2017
## 3:00 - 4:30 PM

AEM 072

**Metatranscriptomic profiles of rhizospheric microorganisms and relationship with canola yield**

Chih-Ying LAY, , T.H. BELL[1], J. TREMBLAY[2], C. HAMEL[3], N. HARKER[4], R. MOHR[5], M. ST-ARNAUD[0], [1]The Pennsylvania State University, [2]National Research Council of Canada, Biotechnology, PA USA

Research Institute, [3]Agriculture and Agri-Food Canada, Québec Research and Development Centre, [4]Agriculture and Agri-Food Canada, Lacombe Research Centre, [5]Agriculture and Agri-Food Canada, Brandon Research Centre. Canola is one of the most important economic crops in Canada. The microbial community inhabiting the rhizosphere of canola may affect crop development, nutrient absorption, and health. Although the root-associated microbial communities for canola have been studied previously, the influence of common agronomic practices on functional gene expression in this community is still poorly known. We studied the effects of high fertilizer application and high seeding rates on functional gene expression, and correlations with canola yield, in canola rhizosphere soils as compared to standard practices and two reference crops (pea and wheat). The field experiment was conducted in Lacombe (AB), Beaverlodge (AB), and Brandon (MB) with a complete randomized block design with four replicates. Sampled from rhizosphere soil samples collected at canola's mid-bloom stage, total RNA was isolated and rRNA-depleted, and reverse-transcribed to double-stranded complementary cDNA for Illumina HiSeq Sequencing. The raw data was filtered for quality and assembled into contigs, which raw reads were mapped against to count the relative read frequencies. Contigs were annotated using the KEGG database to identify the most probable function and biological origin. In all samples, the proportion of bacterial and eukaryotic RNA were ~70% and ~20%, respectively. PERMANOVA was used to test significance of the effects of agronomic practices, crop type, and location on the taxonomic profile of microorganisms at the domain level and their expressed gene profiles. We found a significant effect of crop ($P < 0.001$) on the taxonomic composition of microbial communities, while functional gene expression profiles were significantly influenced by location ($P < 0.001$) and crop ($P = 0.043$). The relationships between canola yield and the genes involved in the nitrogen cycle, phosphorus transportation, plant growth, and pathogen resistance were analyzed using Spearman correlation. Four genes involved in the N-cycle were positively correlated with yield, including *arc*C, *hcp*, *nir*S and *nrt*B. The phosphorus-related genes *pho*R, *man*Z, *man*X, and *pts*H were also positively correlated with yield. For genes potentially related to pathogen resistance, only *bud*C was positively correlated with yield. Genes with a taxonomic identity ≥ 90% belong mostly to *Serratia* and *Pseudomonas*, based on currently available databases. The relative abundance of those genes was significantly higher in Lacombe with the highest canola yields than in the other two locations. Our results suggest that regardless of location, canola yield was associated with elevated microbial expression of genes related to denitrification (*nir*S and *nrt*B), ammonification (*hcp*), P-starvation (*pho*R), P-transfer (*man*Z, *man*X, and *pts*H) and acetoin production (*bud*C), and canola might have a predictable influence on some microbial gene expression.

AEM 112

**Toward antibiotic stewardship: Route of antibiotic administration impacts the microbiota and resistance gene diversity in swine feces**

Nicole RICKER, USDA-ARS National Animal Disease Center, P. COLGAN[1], J. TRACHSEL[2], J.A. JONES[2], A HOWE[1], J.F. COETZEE[3], C.L. LOVING[2], H.K. ALLEN[2], [1]Iowa State University, [2]USDA-ARS National Animal Disease Center, [3]Kansas State University, Manhattan KS

Antibiotic-resistant bacterial infections are a global health crisis, which has resulted in calls for improved antibiotic prudency in both human medicine and animal agriculture. Antibiotics are administered to food-producing animals to treat and prevent disease, and one efficient way to treat a herd is to administer the antibiotic in the feed. In this study, we asked whether route of antibiotic administration differentially impacts intestinal microbial diversity. Given that oral antibiotics would be in direct contact with intestinal bacteria, we hypothesized that oral administration may dramatically impact intestinal diversity when compared to injected antibiotics. We further postulated that these impacts could influence the likelihood of resistance gene acquisition by plasmids and other mobile elements (the mobilome) and therefore impact the diversity and abundance of resistance genes in swine feces. Ten sows were farrowed in environmentally controlled barns, and piglets were weaned at 21 days post-farrow and distributed across three treatment groups. At weaning a therapeutic dose of oxytetracycline was administered either in-feed for 7 days or injected as a single, time-released dose. A third group remained untreated. One-third of the pigs were necropsied on days 4, 7 and 14 post-treatment for collection of ileal and cecal contents and mucosal scrapings. Serum, oral fluids, and feces were collected to monitor concentration of antibiotic by LC-MS/MS. Mobilome content was extracted from day 7 fecal samples using a modified alkaline lysis procedure followed by genomic DNA digestion and multiple displacement amplification and analyzed using long read (Pacific BioSciences) and short read (Illumina) sequencing. DNA was also extracted from intestinal and fecal samples for 16S rRNA gene sequence analysis. Analysis of antibiotic concentrations in the serum revealed that injected antibiotics produced a predictable spike soon after administration which declined but was still present at day 14. Conversely, in-feed administration produced lower but less variable levels of antibiotic in circulation. Bacterial community diversity in the feces was impacted by treatment, with the in-feed treatment group significantly different from both the injected and non-treated groups. Examining the fecal mobilome revealed antibiotic resistance genes in multiple genetic contexts, which will be further queried to evaluate the potential for horizontal transfer of resistance genes. Injected oxytetracycline resulted in a higher concentration of antibiotic in blood, yet showed a reduced disturbance on the gut microbial community, which suggests that route of antibiotic administration may be one critical control point for maintaining healthy gut communities and reducing selection for antibiotic resistance development. These findings may be important not only for disease management in food animals, but also for manure management and antibiotic therapy in human medicine.

II 049

**Mechanistic insights into hierarchal effector secretion in enteropathogenic *Escherichia coli***

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Enteropathogenic *Escherichia coli* (EPEC) adhere to the intestinal mucosa of humans producing attaching and effacing lesions that lead to acute gastroenteritis. EPEC use a needle-like injection apparatus known as the type III secretion system (T3SS) to deliver protein effectors into the host. Secreted effectors allow for intimate attachment with the host cell membrane, cytoskeleton arrangements, increased intestinal permeability, and interfere with normal cellular processes. Previous studies have shown that secretion of the translocated intimin receptor (Tir) requires the chaperone CesT and that the secretion of Tir needs to occur first before the delivery of any subsequent effectors. To understand the molecular interactions driving

hierarchal effector secretion in EPEC we structurally and functionally characterized Tir and CesT. Co-expression and pull-down experiments showed that the minimal CesT binding region of Tir was located to residues 35-77. The structure of this Tir peptide-CesT complex was determined to 2.74 Å revealing a 2:2 stoichiometry, suggesting that Tir may contain two separate CesT binding domains. Using bacterial two-hybrid and co-expression pull-down experiments we locate the second CesT binding region to the C-terminal domain of Tir. Furthermore, secretion assays using various *tir* deletions show that the C-terminal domain of Tir is required for effector secretion. Taken together, the data suggest that CesT dimers deliver Tir to the T3SS in a conformation that allows for N-terminal signal peptide recognition and presentation of the Tir C-terminal domain to the T3SS apparatus that licenses the system for release of additional effector proteins.

II 068

**Immunophage synergy is essential for eradicating pathogens that provoke acute respiratory infections**

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*Pseudomonas aeruginosa*is an important cause of life-threatening nosocomial pneumonia and prone to multidrug resistance. Especially at risk are patients with weakened immunity and chronic respiratory disorders, such as cystic fibrosis. Considering the scarcity of new antibiotics, phage therapy has re-emerged an alternative antibacterial strategy to combat infectious diseases. Several recent animal studies have demonstrated the therapeutic potential of phages for treating pseudomonal lung infection in healthy immunocompetent animals. But, it remains unknown whether phage therapy is effective in hosts with immunodeficiency. Here, we combine an experimental murine model of acute *P. aeruginosa* pneumonia in both immunocompetent and immunodeficient hosts with mathematical modelling, to determine when and how therapeutic application of phage succeeds and when and how it fails. We show that inhaled phage can cure acute pneumonia, as well as provide prophylaxis, in wildtype and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ innate and adaptive lymphocyte deficient hosts. In contrast, phage therapy efficacy was severely reduced in hosts with MyD88 signalling deficiency and lost completely in animals lacking neutrophils. Mathematical modelling the tripartite dynamics between host innate immune response, pseudomonas infection, and phage lysis, predict that innate immune effector cells, particularly neutrophils, are needed to work in concert with the phages reduce phage-sensitive. Neutrophils are also needed to eliminate phage-resistant bacterial cells for treatment to be effective. Interestingly, mathematically modelling without host innate immune response and phage-resistance development, phage treatment on its own would be unable to eliminate bacterial infection in the mouse lungs. This ‘immunophage synergy’ contrasts with the predominant view that phage therapy efficacy relies largely on a phage’s ability to kill bacteria. Mathematical simulations also predict that heterogeneous mixing of phage and bacteria in the lungs, as well as phage saturation, provide barriers and reduce treatment efficacy. Nevertheless, we show that phage therapy can still be efficacious in hosts with weakened innate immunity that manifest emergence of phage resistance, with no untoward immune effects towards the phage.

MGCM 019

**The genome-wide interaction network of nutrient stress genes in *Escherichia coli*.**

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Conventional efforts to describe essential genes in bacteria have typically emphasized nutrient-rich growth conditions. Of note, however, are the set of genes that become essential when bacteria are grown under nutrient stress. For example, more than 100 genes become indispensable when the model bacterium *Escherichia coli* is grown on nutrient-limited media and many of these nutrient stress genes have also been shown to be important for the growth of various bacterial pathogens *in vivo*. To better understand the genetic network that underpins nutrient stress in *E. coli*, we performed a genome-scale cross of strains harboring deletions in some 82 nutrient stress genes with the entire *E. coli* gene deletion collection (Keio) to create 315,400 double deletion mutants. An analysis of the growth of the resulting strains on rich microbiological media revealed an average of 23 synthetic sick or lethal genetic interactions for each nutrient stress gene, suggesting that the network defining nutrient stress is surprisingly complex. A vast majority of these interactions involved genes of unknown function, or genes of unrelated pathways. The most profound synthetic lethal interactions were between nutrient acquisition and biosynthesis. Further, the interaction map reveals remarkable metabolic robustness in *E. coli* through pathway redundancies. In all, the genetic interaction network provides a powerful tool to mine and identify missing links in nutrient synthesis, and to further characterize genes of unknown function in *E. coli*. Moreover, understanding of bacterial growth under nutrient stress could aid in the development of novel antibiotic discovery platforms.

MGCM 046

**CRISPR-Cas: limitations of an adaptive immune system**

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What sets CRISPR-Cas systems apart from other elements of the prokaryotic immune system, and gives them such versatility as a genome-editing tool, is the adaptive nature of the immunity conferred. Despite this, the natural adaptation process by which new immunities are acquired remains the least understood aspect of CRISPR-Cas biology. Adaptive immunity is powerful, but inherently risky: there's the danger of self-targeting (auto-immunity), the relative ease with which it can be bypassed (mutations in recognition motifs), and there's the intrinsic requirement of being exposed to a potentially lethal invader in order to acquire immunity. In exploring the latter, we developed a process to "vaccinate" bacteria using defective bacteriophages, and refined it using plasmids to create "on-demand" immunity in native CRISPR arrays. With these optimized tools, we've been able to uncover new biases in spacer acquisition, as well as investigate a number of factors modulating CRISPR adaptation. In particular, while pre-existing CRISPR immunity is protective over a wide range of phage multiplicities of infection (MOI: phage-to-cell ratio), only a narrow "goldilocks zone" of MOIs permits the ready acquisition of new immunities. Investigating the failure of CRISPR adaptation outside this optimal range has yielded new insights into the evolutionary conditions under which adaptive immune systems are favoured.