Section Symposium Abstracts

Thursday, June 21, 2018
10:30 AM – 12:00 PM

Infection & Immunity

SSII 1

***Staphylococcus aureus* requires the GraXRS regulatory system to adapt to the phagolysosomal compartment of macrophages**

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The macrophage phagolysosome is a highly acidic organelle with potent antimicrobial properties yet, remarkably, phagocytosed *Staphylococcus aureus* replicates within this niche. Herein we investigated what gene(s) allow *S. aureus* to adapt and proliferate within the phagolysosome of the macrophage. We show that the two-component regulatory systems SaeRS and AgrAC, and the a-phenol soluble modulin peptides, all of which are important virulence factors in*S. aureus*, are dispensable for growth in the macrophage phagolysosome. In contrast, we find that the *S. aureus* GraXRS regulatory system is essential for replication of *S. aureus* within this compartment. We next investigated how GraXRS contributes to phagolysosomal replication of *S. aureus*, focusing on the roles of phagosome acidification and antimicrobial peptide exposure in the process. Under acidic conditions *in vitro*, the growth of both wildtype USA300 and its isogenic *graS* mutant is significantly impaired yet, remarkably, we demonstrate that phagolysosome alkalinization is not sufficient to enhance bacterial growth. Together these data suggest that *S. aureus* may need to be exposed to phagosome acidification in order to be able to respond appropriately to this niche within macrophages. To test this notion, we analyzed phagosomes containing replicating *S. aureus* USA300 by fluorescence microscopy and show that replicating bacteria reside within intact phagosomes that are fully acidified. This assertion was confirmed by ratiometric pH measurements of phagolysosomes containing live and dead *S. aureus* USA300 which have an average pH of 5.4. Importantly, through gentamicin protection assays and fluorescence microscopy studies we do find that phagosome acidification plays an important role in modulating the ability of wild-type *S. aureus* to grow within the macrophage. Strikingly, when phagosome acidification was blocked prior to phagocytosis, growth of wild type *S. aureus* was significantly impaired, thereby mimicking the phenotype of a *graS* strain. The notion that acidification is a prerequisite to *S. aureus* growth within phagolysosomes was supported by our *in vitro* growth experiments, whereby exposure to acidic conditions augmented *S. aureus* resistance to antimicrobial peptides and reactive oxygen species in a GraS-dependent manner. In vivo, we show that *graS* and *mprF*, the latter encoding the multiple peptide resistance factor and a member of the GraS regulon, are required for survival in the murine liver during the acute stages of systemic infection when the bacteria reside within liver resident Kupffer cells. We conclude that GraXRS represents a vital regulatory system that functions to allow *S. aureus* to adapt and evade killing, prior to commencement of replication, within host anti-bacterial immune cells.

II 019

Phage Antibiotic Synergy (PAS): A Promising Treatment for Chronic *Pseudomonas* Lung Infections

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Chronic *Pseudomonas aeruginosa* lung infections are characterized by biofilm growth. Biofilms show an increased resistance to antibiotics and treatment of these infections proves problematic when the bacterium has innate multi-drug resistance and the ability to rapidly adapt to its environment. The severe pathogenesis of *P. aeruginosa* makes it the most prevalent pathogen found in the lungs of adult cystic fibrosis (CF) patients. The development of better treatment options is imperative, and phage therapy may be the answer. Bacteriophages (or phages) are viruses that exclusively infect bacteria. Phage therapy, though previously abandoned due to the advent of antimicrobial agents, has begun to resurface due to the careless use of antibiotics creating extreme drug resistant (XDR) bacterial infections that cannot be treated using traditional methods. A phenomenon known as phage antibiotic synergy (PAS) has been observed in which phage activity increases in the presence of some classes of antibiotics at sub-inhibitory concentrations. Additionally, some phages also have the ability to disrupt biofilms, making PAS a promising treatment for biofilm eradication. This study aimed to investigate the synergistic effects between the lytic jumbo phage phiKZ and the prescription inhaled aztreonam antibiotic Cayston™ on *P. aeruginosa* strain PA01. A significant increase in phiKZ plaque diameter in the presence of sub-inhibitory Cayston™ confirmed a synergistic effect exists. Intriguingly, when we increased the sub-inhibitory concentration of Cayston™, the plaque diameter increased accordingly. TEM images show PA01 takes on an aberrant filamentous morphology in the presence of sub-inhibitory Cayston™. Due to their new morphology, pilus function is also affected, causing twitching motility to decrease significantly. Current studies involve treating early and late stage MBEC biofilms to determine if PAS is more effective at clearing chronic infections than phage treatment alone, as well as quantifying phage propagation in the presence or absence of sub-inhibitory Cayston™ to further identify the mechanism in which PAS is occurring. The results from this study show phiKZ activity on *P. aeruginosa* is increased in the presence of Cayston™. Furthermore, the resulting filamentous morphology prevents twitching motility. These findings highlight the need for further research into the use of phage and antibiotics as a potential treatment option for chronic *P. aeruginosa* infections.

SSII3

The polysaccharide biosynthesis genes tviBC are required for capsule production, in vivo fitness and antibiotic resistance in multi-drug resistant Acinetobacter baumannii

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Acinetobacter baumannii, a leading multi-drug resistant nosocomial pathogen infects a wide variety of anatomic sites including the respiratory tract and bloodstream. Although extensive work has been performed to understand the mechanisms mediating drug resistance,relatively little is known about the molecular basis of its pathogenesis. Despite capsule is recognized as a major virulence factor, the molecular mechanisms regulating its biosynthesis in *A. baumannii*is not well understood. Using transposon sequencing (Tn-Seq), we identified genes hypothesized to be involved in capsule polysaccharide biosynthesis, export, and regulation as crucial fitness factors. The polysaccharide biosynthesis genes *tviBC* were among the genes identified. TviBC are involved in the synthesis of UDP-N-acetyl-D-galactosaminuronic acid (UDP-GalNAcA). In *A. baumannii*, it is hypothesized they are involved in capsule and lipooligosaccharides (LOS) biosynthesis. To determine the contribution of *tviBC* in capsule and LOS production, as well as in pathogenesis, a double deletion-mutant of *tviBC* was constructed. As expected, the *tviBC* mutant was deficient in capsule production. Since capsule prevents complement-mediated killing, we tested whether the *tviBC* mutant was more susceptible to human serum than the WT strain. As expected, the mutant was 5.0-logs more susceptible to the bactericidal activity of human serum. Accordingly, in the neutropenic murine model of bacteremia, the

double *tviBC* mutant was unable to colonize the bloodstream. In addition, the *tviBC* mutant showed sign of alteration of its cell envelope integrity since it was unable to grow in the presence of bile salts and was highly susceptible to glycopeptide, carbapenem, aminoglycoside and tetracycline antibiotics. Work is in progress to determine the independent contribution of *tviB* and *tviC* to these phenotypes, as well as in capsule and LOS biosynthesis. In addition to better understand the pathogenesis of *A. baumannii*, these results may also contribute to our understanding of capsule and LOS biosynthesis and ultimately, will allow us to formulate strategies to manage or prevent *A. baumannii* infections.

SSII4

Tracking Phage Through Fecal Microbiota Transfer

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Fecal microbiota transfers (FMTs) have been used to treat human intestinal diseases ranging from *Clostridium difficile* infections to ulcerative colitis. The compositional changes of the gut microbiome following FMT are of considerable interest. Surprisingly, despite the successful outcomes of this therapy, evidence for transplantation of the donor's microbiome remains inconclusive. An often-overlooked component of this transfer is the bacteriophage (phage) population, which could impact the recipient's microbiome. We tracked the highly prevalent gut phage, crAssphage, in samples collected from donors and recipients of FMTs. This phage, originally identified by metagenomics, is present in ~ 40% of humans, although its bacterial host is not yet known. Combining PCR and metagenomic analysis, we were able to observe temporal variability in crAssphage prevalence as well as correlate transfer of the phage with FMTs from phage-containing donors. To assess causality, we also demonstrated engraftment of crAssphage into germ-free mice receiving human fecal matter. We confirmed the presence of an established, replicating phage population. Identifying species transferred into mice alongside crAssphage has enabled us to embark on a targeted approach to culture the elusive host of this remarkable phage.

SSII5

Cephalosporins with improved activity against carbapenem-resistant metallo-β-lactamase-producing Gram negative bacteria

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After more than seven decades since their discovery, β-lactam antibiotics remain key agents for treatment of Gram-negative infections. Among the β-lactams, the carbapenems and third generation cephalosporins are the first choice chemotherapeutic agents. Antibacterial drug resistance is a major clinical problem, particularly due to the reduced efficacy of β-lactam antibiotics against Gram-negative pathogens such as *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Stenotrophomonas maltophilia.* Among the major resistance determinants are zinc-dependent metallo-β-lactamases (MBLs), enzymes which hydrolyze almost all β-lactams including the carbapenems and cephalosporins. We report new cephalosporin derivatives, that bind well to MBLs but exhibit relatively low susceptibility to inactivation by these bacterial enzymes. In particular, cephalosporins bearing an aroylthio group at C3' have been synthesized and evaluated in in vitro enzyme assays with the clinically relevant MBLs IMP-1, VIM-2, NDM-1, SPM-1 and L1 as substrates well as inhibitors of the hydrolysis of carbapenems by MBLs. Two mechanisms for MBL inhibition, appear to be operative. In mechanism A, the initial hydrolysis product undergoes a rapid expulsion of the 3-aroylthiobenzoate group before product release from the active site such that the cephalosporic acid product is converted into a metal binding agent that is released only slowly by the MBL. In mechanism B, the aroylthiobenzoate anion that was expelled from the active site returns to bind to the active site zinc ions. Enzyme inhibition appears to occur largely via mechanism A for most MBLs;

however, inhibition of L1 appears to result from contributions from both mechanisms A and B. In in vitro experiments involving the hydrolysis of meropenem by clinically important MBLs, the half-life of this carbapenem has been found to be extended the very substantially in the presence of these cephalosporin derivatives. Additionally, some of these cephalosporins are found to act synergistically with meropenem against MBL producing Gram negative clinical isolates. One of these cephalosporins, UW123, has been found to exhibit substantial intrinsic antibacterial activity against Gram negative bacteria including MBL-producing clinical isolates in vitro. Time-kill experiments suggest that UW123 is bacteriostatic. Penicillin binding protein (PBP) assays using the fluorescent bocillin as a probe reveal that UW123 bind predominantly to PBP 1a/1b, PBP2 and PBP3 in*E. coli* and to PBP1b, PBP1c, PBP3 and PBP4 in *Pseudomonas aeruginosa.* In cell-based toxicity experiments, UW123 has been found to be non-toxic up to 375 mg/L. UW123 is, however, susceptible to inactivation by serine-type extended spectrum beta lactamases (ESBLs) such as CTX-M-15. Since clinical isolates can produce both MBLs and SBLs, practical applications of such cephalosporins with need to be done in combination with SBL inhibitors. UW123 and related cephalosporins are promising candidates for potential development into useful antibiotics or adjuvants to improve therapy with MBL-susceptible β-lactams such as carbapenems.

II 015

Development of a "National MALDI Database" to enable Provincial Laboratories to Identify Rare and Novel Clinical Bacterial Isolates Using MALDI-TOF MS Analysis

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Rapid and accurate diagnosis of infectious diseases is required for effective treatment of patients and to perform routine surveillance for common nosocomial or community-acquired pathogens. Traditional methods of identification of bacteria, yeast and filamentous fungi, in clinical laboratories, involve the use of biochemical and phenotypic testing, as performed at many hospital and community-based private laboratories in Canada. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a revolutionary tool for the rapid and cost-effect identification of microorganisms in clinical diagnostic laboratories. This method is used to determine microbial identification by determining the unique 'protein spectrum fingerprint' for each sample and it performs equally to, if not better than, traditional biochemical-based identification methods. However, MALDI users rely on proprietary commercial databases to identify these microbes, where several organisms of public health importance are not represented in these commercial databases due to their rarity and will not be detected nor correctly identified using the MALDI-TOF MS method. Furthermore, there is evidence that custom MALDI databases created using local isolates provide more accurate identifications to species level compared to using commercial MALDI databases. Thus, there is a clear need to create a supplementary database containing species missing or underrepresented in commercial spectral databases. This project aims at developing and deploying a "National MALDI Database" (NMD) that is initially being populated using spectra of select isolates from the National Microbiology Laboratory's vast collection of well-characterized bacteria, one of the largest and most extensive collection of bacterial isolates in Canada containing common and rare/unusual/novel bacterial pathogens that are not typically found in the commercial spectral databases. Isolates were processed using standard formic acid:ethanol extraction protocol, and the spectra for each isolate were generated using the Bruker Daltonik Microflex MALDI-TOF MS. To date, the spectra of 746 species, either absent from or underrepresented in the Bruker Biotyper commercial database, has been added to the NMD, and successfully beta tested independently by five external sites, with an end goal of 800-1000 species being shared in the final version of the NMD. A major outcome of this project is the provision of the NMD as a centralized repository of valuable spectra data to be shared across the extended network of Canadian public health and diagnostic laboratories, leading to rapid identification of potential human pathogens by diagnostic

laboratories locally, thereby providing more focused therapies, shorter hospital stays, decreased mortalities, and overall enhanced public health outcome.

Applied & Environmental Microbiology

SSAEM1

Response of P-cycling microbial communities during soil genesis and ecosystem development along a 6500 year chronosequence under lowland temperate rainforest in New Zealand

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The Haast chronosequence (SW coast, South Island, NZ) comprises a series of dune ridges formed by sediments deposited following periodic earthquakes on the Alpine Fault. It is characterized by rapid podsolization under high rainfall and has proven to be an ideal system to study soil organic P dynamics because of a rapid depletion of mineral P and accumulation of organic P over pedogenesis. The total P decline at Haast is associated with shifts in soil organic P composition, plant communities and bacterial diversity. The importance of the microbial contribution to organic P turnover under P limited conditions is well established, and prior isotopic studies at Haast pointed to the importance of phosphatases in organic P mineralization in the older dunes. Our recent development of PCR primers targeting three classes of bacterial non-specific acid phosphatase genes (Class A, B and C NSAPs), enabled the exploration of the microbial contributions to soil P dynamics in these long-term and highly acidic soil systems. Six dune ridges were chosen for the study (ranging from 191 years before present to 4000 yBP). Mineral soil samples (n=6) from each dune ridge were collected and immediately placed into Lifeguard™ Soil Preservation Solution to preserve nucleic acid integrity until total DNA and RNA could be extracted. Initial measurements targeted total bacteria (16S rRNA) and Class A, B and C NSAPs by quantitative PCR. Total bacteria decreased over time following ecosystem retrogression, as did the class A and class C NSAP targets. Interestingly, the Class B NSAP gene was rarely detected; although we have successfully quantified this group in NZ pasture soils. Relative to total bacteria, the NSAP targets were higher in the oldest dunes and lower in the youngest dunes, suggesting a shift in the microbial composition to more phosphorus solubilizing bacterial communities over time. Finally, as found with the bacterial phosphatase genes, potential acid phosphatase activity (ACP), increased on average over time through retrogression. PCR amplicons targeting total genes and gene transcripts will be sequenced using Illumina MiSeq to characterize shifts in the diversity and activity of the bacterial phosphatase producing communities through ecosystem development. This study shows that molecular analysis targeting key functional genes can provide a useful tool for researchers to obtain insight into the role of microbes in biogeochemical cycling.

SSAEM2

Production of alginate lyases by the bacteria isolated from seaweed wastes

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The changes in climate and land use practices have been frequently generating brown algal blooms in coastal waters of the world's oceans. Alginate (a linear anionic polysaccharide of α-L-guluronic and β-D-mannuronic acid residues) is the major structural component of brown algae. Considerable efforts have been directed worldwide toward the development of environmentally friendly methods for the transformation of polymeric alginate into value-added products, including food additives, biofertilizers, biofuels, animal feeds, cosmetics and pharmaceuticals. Physicochemical methods, including alkaline wet oxidation, acid hydrolysis, hydrothermal, and various ionic liquid treatments, have been used for the extraction and processing of alginate from the algal biomass. However, the production of secondary pollution from these techniques necessitates the development of enzymatic methods for the recovery and deconstruction of alginate. Alginate lyases (EC 4.2.2.3 and EC 4.2.2.11) are the key enzymes that cleave alginate into uronic acids-containing oligosaccharides via β-elimination of 1,4-glycosidic linkages. In recent years, microbial biocatalysts harboring alginate lyase activities have been emerging as the robust ecofriendly tool for the bioprocessing of alginates. During the search for industrially important enzymes from the bacterial associates of brown seaweed wastes accumulated off the coast of Barbados, several potent alginate lyase-producing bacteria have been isolated, using serial dilutions and spread plate techniques on mineral medium supplemented with sodium alginate. The rapid screening for the alginate lyase activities in these bacterial isolates was conducted via the Gram's iodine staining method. Phylogenetic affiliations of the selected isolates were determined using the 16S rRNA gene sequencing approach. The enzymatic properties of the selected alginate lyase-producing bacterial isolates indicate the potential use of these isolates as biocatalysts for the biotransformation of polymeric alginate into biologically active oligosaccharides.

SSAEM3

Shining light on phototrophic bacteria: a syntrophy of teamwork for hydrocarbon degradation

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End-Pit Lake (EPL) research is of paramount importance for determining the success and sustainability of reclamation and remediation strategies being considered for the oil sands mines in Northern Alberta, Canada. EPLs incorporate mine wastes (waters, clays, sands and residual hydrocarbons), natural waters, and sediments within open-mine pits for the purpose of remediating and reclaiming once scarred landscapes into natural and sustainable ecosystems. However, given the short existence of the only existing EPL (Base Mine Lake) and multiple planned but not initiated EPLs, little is known about the possible long-range ecological integrity of this reclamation strategy. In particular, even less is known about the functional capabilities of the indigenous microbial populations within the oil sands region and in freshwater boreal lakes in general. Using two, 43-year-old reservoirs (not impacted directly by industry) as surrogate EPLs, we use in-situ metatranscriptomics analysis to piece together a unique syntrophy of natural microbial activity. This is the first microbial study to provide some understanding of the long-term outcomes of EPLs and their ability to degrade contaminates. Results show a strong phototrophic presence in a mixed consortia biofilm at water depths down to 10-12 m. The syntrophic cooperation observed, between phototrophs and chemotrophs, is providing efficient mechanisms to control the biodegradation of measured hydrocarbons within the bed sediments. These truly community-based observations advance our understanding of freshwater, sub-oxic ecosystems, and provides a future glimpse into the microbial function and biodegradation abilities of hydrocarbon influenced EPLs.

SSAEM-4

RNA-Seq of *Delftia acidovorans* RAY209 during colonization of canola roots reveals strong plant-growth promoting behaviour

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Delftia acidovorans is a common environmental β-proteobacteria isolate, with strains identified for their potential in plant-growth promotion and bioremediation activities. *Delftia acidovorans* RAY209 (RAY209) has been shown to associate with and improve yield of economically important cops of canola and soybean. To investigate the mechanisms by which this isolate interacts with canola roots canola seedlings were grown hydroponically in Hoagland's medium and inoculated with RAY209. Seven days post-inoculation, mRNA from the bacteria associated with the root-surface and in the plant-medium were purified, depleted of bacterial and plant rRNA and cDNA was DNA sequenced. Analysis of the RNA expression profiles from root-associated and planktonic cells reveals several metabolic adaptations of RAY209 to an epiphytic lifestyle. Among these include metabolism related to siderophore and auxin (plant-horomon) production and polyhydroxybutyrate(PHB) synthesis. Relative to planktonic cells, several ABC transporters, including ones specific for carbohydrates and metals, were also differentially expressed in root-associated cells. In addition to confirming RAY209's plant-growth promoting capacity, and providing insights into this isolate's rhizosphere colonization strategy, the novel experimental growth system established for RAY209-inoculated canola will allow for further testing of biological questions related to the epiphytic lifestyle of RAY209.

SSAEM5

HMMER or SPAdes? Informing bioinformatics tool choice with a systematic comparison of microbiome methods

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The sequencing of taxonomic markers such as the 16S ribosomal RNA gene is a widely used method for culture-independent surveying of microbial communities. Over the past several years, there has been a significant increase in the number of bioinformatics tools available to process marker gene sequences. These can come in the form of comprehensive software suites like QIIME and mothur that provide an entire pipeline, or more specific tools such as vsearch or CD-HIT for operational taxonomic unit clustering. The correct choices for pipeline, tools, and parameters are often not obvious, and the optimal approach may depend on the specific dataset being used. For example, using the Ananke time-series clustering software we have recently demonstrated that the 97% OTU clustering cut-off can aggregate too aggressively and obscure key temporal dynamics, suggesting a denoising approach, such as DADA2 or deblur, is preferred for some data sets. Here we propose a framework for a systematic, automated analysis of marker-gene pipelines. Each step in these complex workflows affects the downstream results, and our framework aims to tease out the impact that each computational method and parameter choice has on the inferred community statistics. We will use sequenced mock communities, available through the "mockrobiota" project (https://github.com/caporaso-lab/mockrobiota), and simulated microbial communities to analyze the impacts of various methods and parameters on the resulting community statistics. In addition, we will assess the degree to which input temporal patterns can be recovered by a given bioinformatic pipeline using the Ananke time-series clustering software. Our benchmarking platform will be based on the nucleotid.es platform, which was developed as a web-based solution for automated benchmarking of metagenome assembly tools. Compute container solutions (such as Docker) allow the complete bioinformatic pipelines to be faithfully reproduced on any system. Our platform will allow researchers to investigate the practical impacts that highly debated procedures such as OTU clustering, sample rarefaction, and reference database trimming have on their downstream results. Our platform aims to provide a continually updated resource that helps the

microbiome research community compare and evaluate the tools available in the rapidly expanding software ecosystem.

SSAEM6

Drinking Water Security in Manitoba First Nations Communities

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The NSERC CREATE H2O program collaborated with a number of First Nations communities in Manitoba to evaluate the levels of bacteria in water distribution systems and assess community members concerns and management of their household drinking water. This included taking water samples from homes relying on piped water, below-ground concrete or fibreglass cisterns, above-ground polyethylene cisterns stored in insulated shelters, and from buckets/drums in homes without running water. The main findings of the research are that despite well functioning water treatment plants in communities, the tap water in many First Nations homes relying on below-ground concrete cisterns and buckets/drums contained fecal bacteria at alarmingly high levels. Antibiotic-resistance genes were also detected in a range of drinking water samples. Some household's report having to frequently buy bottled water to ensure their family's drinking water supply is safe, and/or to having to ration their water in order to have enough for basic personal and domestic water needs. The issue of poor drinking water quality in First Nations communities in Canada remains unsolved and, to protect human health and well being, there is an urgent need for improved monitoring and upgrading of infrastructure in communities relying heavily on under-ground cisterns and standpipes for drinking water.

Molecular Genetics & Cellular Microbiology

SSMGC1

SCAB79581 is a ThiF family protein that promotes coronafacoyl phytotoxin production and virulence in the common scab pathogen *Streptomyces scabies*

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Streptomyces scabies is an important causative agent of potato common scab, which is one of the most challenging diseases affecting potato production in Canada. Among the known or potential virulence factors that are produced by *S. scabies* is coronafacoyl-L-isoleucine (CFA-Ile), which is a member of the coronafacoyl family of phytotoxins that contribute to the virulence phenotype of several different phytopathogenic bacteria. The production of CFA-Ile in *S. scabies* is controlled by the PAS-LuxR family regulator CfaR, which functions as a positive activator of the metabolite biosynthetic genes. Downstream of the *cfaR* gene is a gene designated *SCAB79581*, which encodes a predicted ThiF family protein of unknown function. Previous work showed that *cfaR* and *SCAB79581* are co-transcribed, suggesting that *SCAB79581* may also function in the regulation of CFA-Ile biosynthesis. The objective of this study, therefore, was to further investigate the role of *SCAB79581* in the biosynthesis of CFA-Ile in *S. scabies.* A Δ*SCAB79581* deletion mutant was generated in *S. scabies* by replacing the gene with an antibiotic resistance cassette. In addition, *S. scabies* strains were constructed in which the *cfaR* and/or *SCAB79581* gene was overexpressed from the strong, constitutive *ermE*p* promoter. The production of CFA-Ile in the different strains was tested by analyzing culture extracts by high performance liquid chromatography (HPLC). In addition, a potato tuber disk bioassay was performed in order to assess the virulence phenotype of the overexpression strains, while gene expression in the strains was tested using semi-quantitative RT-PCR. Analysis of culture extracts using HPLC showed that the Δ*SCAB79581* strain was reduced in CFA-Ile

production as compared to the control strain, though some production could still occur. Overexpression of *cfaR* alone and *cfaR+SCAB79581* significantly enhanced CFA-Ile production levels, with the latter strain producing the greatest levels. In contrast, overexpression of *SCAB79581* alone did not significantly affect the production of CFA-Ile. Semi-quantitative RT-PCR confirmed that overexpression of *cfaR+SCAB79581* leads to higher expression of the CFA-Ile biosynthetic genes as compared to both the control and the overexpression of *cfaR* alone. Furthermore, virulence bioassays demonstrated that both the *cfaR* and *cfaR+SCAB79581* overexpression strains caused greater necrosis and pitting of potato tuber tissue as compared to the control strains, with the latter producing the most severe disease symptoms. The results of our study show that *SCAB79581* functions together with *cfaR* to activate CFA-Ile biosynthetic gene expression and metabolite production in *S. scabies*. Importantly, our study also provides further evidence supporting a role for CFA-Ile as a virulence factor in *S. scabies* pathogenicity.

SSMGC2

Revealing antimicrobial resistance gene mobility trends using >15000 replicons

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Antimicrobial resistance (AMR) is an emerging issue that has not been effectively addressed worldwide. In Canada, hospital-acquired AMR infections are approaching 20,000 per year and global AMR related death is 700,000 annually. Advances in genomics has transformed infectious disease surveillance and improved assessment of public health risks associated with AMR. Nevertheless, limitations still exist in understanding the mobility of resistance genes between bacteria species, which is needed for better and more focused risk assessment of AMR spread in pathogens of both human health and agri-foods interest. To date, some AMR genes have been empirically observed to be highly mobile, contributing to clinically-relevant resistance genes that appear to circulate between pathogens. Despite these observations, no large-scale study has ever shown if AMR genes are strongly associated with mobile elements, including plasmids and genomic islands comprising phage, integrons, transposons etc. Here, we present the first comprehensive examination of trends in AMR association with mobile elements across all bacterial species sequenced to date (~16600 bacterial genomes and plasmids from NCBI refseq, as of Jan 2018). AMR profiles for deduced proteins from these replicons were predicted using the Resistance Gene Identifier (RGI) and genomic islands (GIs) were predicted using IslandViewer 4. The AMR gene dataset was combined with the GI predictions and plasmids to find AMR genes that overlapped these mobile elements and tested for significance of association. This first large scale mobility analysis reveals that AMR genes, collectively, are disproportionately found in mobile elements including GI and Plasmids. However, classification of AMR genes into higher-level categories (e.g. resistance mechanisms) using the Antibiotic Resistance Ontology (ARO), identifies certain drug classes and resistance mechanisms that are significantly more associated with GIs or plasmids (for example, beta-lactamases). Notably, passive resistance mechanisms that do not effect the environmental antibiotic concentration (surrounding the bacterial cell encoding the resistance) do not tend to be mobile, and we propose an evolutionary model that would predict which AMR genes may be more likely to be horizontally transmitted. The analyses presented here are an important step in gaining perspectives on global trends of AMR transmission across diverse environments. Identifying classes of resistance that are associated with mobile elements is of interest to both public health and the agri-foods industry which are concerned about the risk of AMR spread between animals, food products and the environment, as well as impacts on the ability to use certain front-line drugs against infections. These results could aid investigations of risk assessment of AMR transmission and aid prioritization of policies for different antimicrobial classes regarding appropriate antimicrobial use.

SSMGC4

The PhoPQ two-component regulatory system controls omptin protease activity in enterobacteriaceae

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In order to resist the innate antimicrobial defences of the host, bacteria have evolved specific mechanisms that enable them to colonize and/or invade mucosal surfaces. In enterobacteriaceae, the PhoPQ two component signaling system senses the presence of cationic host-defence peptides leading to the production of lipopolysaccharide with both increased hydrophobicity and more neutral change, thereby promoting resistance to this class of molecules. In addition to altering cell surface charge and hydrophobicity, enterobacteriaceae also express a number of different omptin proteins, include OmpT, OmpP and ArlC in *Escherichia coli*, PgtE in *Salmonella enterica*, and CroP in *Citrobacter rodentium*. Omptins are a family of outer membrane proteases that have been linked to increased resistance to α-helical host-defense peptides like CRAMP and LL-37 and previous work has shown that omptin activity is associated with increased virulence in several animal models of infection. Here, we show that omptin activity in all of these species is increased by growth in PhoPQ inducing conditions. Furthermore, we show that deletion of the PhoPQ regulatory system results in complete loss of omptin activity in *E. coli*, *S. enterica*, and *C. rodentium*, suggesting that the PhoPQ system is involved in either the direct or indirect regulation of these proteins. Using a combination of western blotting and translational GFP fusions, we show that regulation of the OmpT protease depends on the PhoP transcriptional regulator. We identified a putative PhoP-binding site in the *ompT* promoter and, using site-directed mutagenesis, we show that mutation of this site results in complete loss of *ompT* transcription. We propose a model whereby the PhoPQ system is required for the expression of diverse omptin alleles in multiple species of enterobacteriaceae.

SSMGC5

The lysogenic conversion of *Stenotrophomonas maltophilia* strains by temperate bacteriophages DLP3 and DLP4

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Stenotrophomonas maltophilia is an environmentally ubiquitous Gram-negative bacterium that is emerging as a nosocomial pathogen associated with opportunistic infections in immunocompromised individuals. Treatment of *S. maltophilia* infections are difficult due to high levels of innate antibiotic resistance. Bacteriophages are known to contribute to the acquisition of antibiotic resistance genes and a large proportion of *S. maltophilia* bacteriophages characterized to date encode moron genes (~36%). Thus, studying bacteriophages of *S. maltophilia* could provide insight into the role of phages in antibiotic resistance and virulence trait acquisition of clinical isolates. Soil enrichment using clinical strains D1571 and D1585 enabled isolation of temperate phages DLP3 and DLP4 respectively. Paired-end Illumina MiSeq reads were assembled with SPAdes and resulting genomes were annotated with Glimmer and GeneMark.hmm. Putative functions were assigned with BLASTp, Phyre and I-TASSER. Final genomic assembly of DLP3 resulted in a ~43.6 kb genome encoding 59 open reading frames (ORFs) and two tRNAs. This contrasts DLP4, which has a ~64 kb genome encoding 83 ORFs and one tRNA. Both phages were found to contain putative antibiotic resistance proteins and virulence factors. Two proteins of interest encoded by DLP3 identified for further study were ErmC and a Zmp1-like protein. The ErmC protein is a rRNA methylase that confers resistance to erythromycin. Minimum inhibitory concentration (MIC) experiments with D1571 and D1571::DLP3 show higher inducible resistance levels in the lysogen compared to wildtype, indicating a functional ErmC. The second protein of interest is a putative zinc-dependant metalloprotease similar to the secreted Zmp1 from *Clostridium difficile.* The functionality of the putative metalloprotease is currently under investigation. Two proteins encoded by DLP4, dihydrofolate reductase and N-glycosidase YbiA, were investigated for their functionality. The DLP4 *dhfr* gene was expressed in *Escherichia coli* DH5α for MIC experiments as *S. maltophilia* D1585 is highly resistant to trimethoprim. Expression of *dhfr* lead to a 260-fold increase in

trimethoprim resistance compared to empty vector control in DH5α. The YbiA protein was studied for its ability to restore the swarming phenotype of *E.coli* BW25113 *ybiA*$^{-}$. Expression of DLP4 *ybiA* was found to only partially complement the *ybiA*$^{-}$ mutant. Although the phenotype of D1585::DLP4 is relatively unchanged compared to wildtype, RT-PCR confirmed both genes are expressed during the lysogenic cycle. In summation, both bacteriophages encode functional antibiotic resistance proteins and DLP4 was confirmed to encode a partially functional virulence factor. These results suggest *Stenotrophomonas maltophilia* bacteriophages play an important role in antibiotic resistance and virulence trait acquisition of *S. maltophilia*.

SSMGC6

Type VI Secretion System Dynamics and Modeling

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Bacteria overtake their competitors utilizing a diverse range of strategies. The type VI secretion system (T6SS) is a contractile needle-like organelle that propels toxin effectors directly into the cytosol of surrounding cells. The T6SS needle is anchored to the cell envelope by a cytosolic baseplate and a membrane-spanning complex. Conformational changes in the baseplate are believed to initiate sheath contraction. However, the signals that define polymerization dynamics are unknown. Here we investigated the mechanisms that regulate T6SS polymerization in the Gram-negative bacterium *Vibrio cholerae*.Using a spheroplast model to image the dynamics of the T6SS contraction, we found that the T6SS could undergo similar contraction and assembly process as observed in untreated cells. However, the T6SS needles displayed high variation in lengths that is likely dependent on the orientation of the initial polymerization. This was also observed using non-contractile needle mutants. Our results highlight the flexibility of the T6SS length and lead us to propose a conceptual model for the dynamic process of T6SS firing and recycling.

SSMGC3

Molecular basis for immunity recognition of a type VII secretion system exported antibacterial toxin

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Gram-positive bacteria employ the type VII secretion system (T7SS) to facilitate interactions between eukaryotic and prokaryotic cells. In recent work, we identified the TelC protein from *Streptococcus intermedius* as an antibacterial effector that mediates T7SS-dependent interbacterial competition by degrading peptidoglycan precursor lipid II in the periplasmic space of target cells. Intercellular intoxication of sister cells does not occur because recipient cells express the TipC immunity protein, which inhibits the lipid II phosphatase activity of TelC. In the present study, we used X-ray crystallography to determine the 1.9Å structure of TipC, which shows that this protein adopts a crescent-shaped mixed a/bfold. Using structure-guided mutagenesis, we find that the concave surface of TipC is required for its TelC-specific inhibitory activity. Subsequent sub-cellular localization and bacterial toxicity assays demonstrate that TipC possesses an N-terminal transmembrane helix that facilitates its localization to the plasma membrane and places the TelC-inhibitory domain outside of the cell. While *S. intermedius* strains lacking the *tipC* gene are susceptible to growth inhibition by TelC delivered between cells, we find that the growth of these strains is unaffected by endogenous or overexpressed TelC even though the toxin accumulates in culture supernatants. Together, these data indicate that the TelC-inhibitory activity of TipC is only required for intercellularly-transferred TelC and that the type VII secretion apparatus transports TelC directly from the cytoplasm of the toxin-producing cell to either the extracellular milieu or target bacteria, bypassing the cellular compartment in which it exerts toxicity en route.