Speakers' Abstract

Note:

IS: Invited Speaker | **AEM:** Applied Environmental Microbiology

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

SYMPOSIUM I: Microbial Diversity
Wednesday, June 21st, 2017
8:30 -10:00 AM
(Unified Theme)

IS 002
Invisible Influence: The Microbiome and Human Health
Jack GILBERT, University of Chicago, Chicago IL

The human microbiome is quickly being recognized as a dynamic part of the human ecosystem, and research is starting to demonstrate that using ecology to understand this ecosystem has profound benefits for patient wellness. The immune system controls our interaction with the microbial world, and yet the microbial communities in our bodies are central to modulating the immune response. Changes in the human microbiome have substantial influence on atopy, neurological disorders, metabolic disorders, and a range of complex conditions and disease states. We will discuss evidence of these mechanisms of interaction and how we have started to disturb the delicate balance of the immune-microbe equilibrium, impacting the development and function of our immune systems. Central to this disturbance is the distance we have placed between our children and the microbial world, which has been demonstrated to have a substantial influence on their physiological, immunological, neurological and even endocrinological development. We are now able to significantly reduce cows milk allergy in infants through active manipulation of the gastrointestinal microbiota. We can also reduce surgical infections by feeding the microbiome, preventing virulence activation, and reduce sepsis by using the microbiome to stimulate immune activation. Applying new strategies to identify how the microbial ecosystem correlates with diseases states and treatment efficacy through Microbiome-Wide Association Studies (MWAS) is altering the trajectory of precision medicine, and providing a new framework for facilitating patient care.

II 075

Structural and Functional Characterization of Environmental Antibiotic Resistance Enzymes Reveals New Mechanistic Diversity

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Antibiotic resistance is a serious and growing problem impacting all classes of antibiotics currently in clinical use. One of the main molecular mechanisms of resistance is mediated by bacterial enzymes catalyzing chemical modification of antibiotic molecules, thereby reducing their affinity for their cellular targets. Genes encoding these enzymes are known to be shared, or related in ancestry, between clinical and environmental bacterial species, thus defining a global antibiotic "resistome." Since most of the resistome remains uncharacterized, it is important to develop a pre-emptive understanding of this reservoir of new antibiotic resistance genes before their ultimate dissemination into pathogens. In this study, we characterized the molecular structures, antibiotic substrate specificity and enzymatic properties of novel aminoglycoside acetyltransferases isolated from metagenomic surveys of grassland soils. Our analysis demonstrates that these enzymes (which we term meta-AACs) are new members of Gcn5-Related-N-Acetyltrasferase structural family and are able to confer resistance to aminoglycosides gentamicin C, sisomicin and tobramycin. Moreover, the meta-AAC0020 enzyme demonstrated enzymatic activity comparable with its closest AAC enzyme relative isolated from a clinical isolate of Pseudomonas aeruginosa. The crystal structure of meta-AAC0020 in complex with sisomicin confirmed the AAC(6') regiospecificity of this enzyme while revealing the drug binding mechanism distinct from previously characterised AACs. This illustrates that environmental antibiotic resistance enzymes possess new properties that may result in clinical complications if ultimately mobilized into pathogens. Together our data highlights the presence of highly active and novel antibiotic modifying enzymes in environmental microbiome that has to be taken into account in our quest for novel antimicrobial therapies less prone to resistance.

AEM 126

Sand harbours distinct communities of attached and interstitial bacteria and protists

Vera TAI, University of Western Ontario, N OKAMOTO[1], PJ KEELING[1], [1]University of British Columbia

Sandy beaches dominate coastlines worldwide and serve as buffers between land and sea. These are dynamic environments composed of shifting sediments and variable mixtures of marine and freshwater due to tidal and seasonal storm cycles. To better understand the contributions of the microbial community to sandy beach ecosystems, we investigated the diversity of protists and bacteria from five sandy pocket beaches on the northwest corner of Calvert Island, located in the central coast of British Columbia, Canada by sequencing variable regions of the small subunit ribosomal RNA gene (V4 16S rRNA for bacteria and V4 and V9 18S rRNA for protists). Sand was sampled from different elevations along transect lines from the high tide line to the swash zone. As expected, we uncovered rich microbial communities that likely contribute to

II 082

Culture-enriched metagenomic sequencing of the cystic fibrosis lung microbiota.

Fiona WHELAN, McMaster University, B WADDELL[1], SA SYED[2], HR RABIN[1], MD PARKINS[1], MG SURETTE[2], [1]University of Calgary, [2]McMaster University, Hamilton ON

Next-generation sequencing technologies have afforded the field of microbiome research with the ability to profile communities without the need to culture their inhabitants. While the power of these technologies is clear, combining these approaches with classical, culture-dependent methods can provide us with greater resolution of human microbiota. In this work, we have combined advances in marker gene and metagenomic sequencing with culture-enriched molecular profiling to study the microbiota of the cystic fibrosis (CF) lung. Culture-enrichment consistently recovered the CF lung microbiota, culturing an average of 81.21% of OTUs representing 99.15% of the relative abundance of those sequenced from sputum lung samples directly. Further, culture-enrichment retrieved 65.5% more species than culture-independent methods, reflecting the selective ability of media to enrich for low abundance organisms. Using a novel plate coverage algorithm, we conducted metagenomic sequencing on a minimal subset of culture plates in a patient-specific manner. When compared to culture-independent methods, culture-enriched metagenomics consistently recovered more organisms with better genome coverage when compared to metagenomics conducted on sputum samples. Culture-enrichment of sputum reconstructs the CF lung microbiota for a better understanding of this disease.

AEM 025

When in Drought: the Root Microbiome under Water Stress

Fitzpatrick CONNOR, University of Toronto Mississauga, J COPELAND[1], DS GUTTMAN[1], PM KOTANEN[2], P WANG[1], MTJ JOHNSON[2], [1]University of Toronto, [2]University of Toronto Mississauga ON

Plants have interacted with soil microbes since their colonization of terrestrial environments450 million years ago. Of particular importance are the microbes in close association with plant roots – the root microbiome. As a result of active and passive physiological processes plants recruit a unique community from the enormous pool of soil microbial diversity to colonize their root exterior (rhizosphere) and interior (endosphere). The root microbiome is thought to play an important role in numerous aspects of plant health. Here we focus on the compositional changes occurring in the root microbiome under drought conditions. We combined manipulative experiments with deep amplicon sequencing to understand: 1) how the root microbiome varies across a diverse group of plant species; 2) drought influences the root microbiome across numerous plant species. In total we sequenced nearly 650 rhizosphere, endosphere, and bulk soil samples collected from replicate individuals of 30 plant species grown in well-watered and drought conditions. We found consistent differences between the diversity and composition of endosphere and rhizosphere communities. However, within each community type we uncovered a tremendous amount of variation in both the diversity and composition across plant host species. Evolutionary relatedness among plant hosts was significantly correlated with similarity in the diversity and composition of the endosphere, but not rhizosphere. We found enormous effects of drought on root microbiome composition, but only weak effects on diversity. Surprisingly, the endosphere exhibited a larger shift in response to drought than the rhizosphere. Remarkably, we found that plant host species exhibited little variation in their root microbiome shift in response to drought. In particular, members of certain bacterial families within *Actinobacteria* had the greatest enrichment in plant roots under drought stress. We provide the most comprehensive sampling of the root microbiome across plant species to date. Plant species vary in the composition and diversity of their endosphere communities, which is partly driven by the evolution of traits related to primary productivity and root morphology. Despite this variation occurring across plant hosts, we have uncovered a conserved response of the root microbiome to drought, a globally important environmental stress for plants. Our current

work focuses on understanding whether this conserved drought response represents adaptive plant plasticity mediated by the root microbiome.

SYMPOSIUM II: Microorganisms and Ecosystem Services
Wednesday, June 21st, 2017
8:30 -10:00 AM
(AEM)

AEM 001

Identifying and quantifying microbial interdependencies that underpin ocean productivity

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Marine phytoplankton are responsible for roughly half of global net primary production, support marine food webs and play critical roles in the global carbon cycle. Phytoplankton often require exogenous sources of specific organic and organometallic micronutrients for growth; these compounds appear to originate largely from co-occurring microbial communities. In some cases, the availability of these compounds can limit the growth of phytoplankton or drive changes in community composition. The most well-characterized examples in marine systems are cobalamin (vitamin B_{12}) and thiamine (vitamin B_1). Recent developments suggest that there are a series chemical transformations of micronutrient-related compounds, conducted by different, co-occurring marine microbial groups, which result in a broad chemical repertoire of nutritional factors that can support primary production. This chemical repertoire is poorly characterized and not currently predictable from genomic information. Here we identify two specific examples of these interactive, microbially-mediated chemical transformations of micronutrients and present hypotheses about the key microbial groups involved. We then utilize proteomic and transcriptomic approaches to identify and quantify the role of these micronutrients and their transformations in sustaining marine primary production from the North Atlantic to the Southern Ocean.

AEM 137

Ecological Perspectives on the Degradation of Lignocellulosic Polymers by Bacteria and Fungi in Forest Soil

Roland WILHELM, University of British Columbia, R SINGH[1], H LEUNG[1], LD ELTIS[1], WW MOHN[1], [1]University of British Columbia

The capability of various bacterial isolates to degrade each of the three major components of lignocellulose (lignin, cellulose and hemicellulose) has been reported since the 1970s. Attention is now focused on improving our ecological understanding of these, and other yet-to-be cultured lignocellulose degraders, in their environment where degradation is a multifaceted process performed by a consortium. The technique of ^{13}C-stable isotope probing allows for the identification and characterization of lignocellulolytic populations in a cultivation-independent manner, maintaining a semblance of their environmental context. We surveyed the composition and activity of lignocellulolytic populations in forest soils from across North America by incubating the soils with ^{13}C-labeled model polymers for all three components of lignocellulose. Community-level traits and the genomic content of individual taxa were determined by shotgun and amplicon sequencing of ^{13}C-enriched DNA, while total activity was measured by quantifying ^{13}C-enrichment of DNA and phospholipid fatty acids (PLFA). We found that few bacterial taxa incorporated ^{13}C from all three polymers, though the capacity to use all three was conserved within highly active families of

Burkholderiales (Betaproteobacteria) and genera of Caulobacteraceae (Alphaproteobacteria). Conversely, fungal taxa commonly possessed the capacity to degrade all three polymers. The activity of Gram-negative bacteria surpassed the activity of fungi on cellulose and lignin in most soils, and persisted in fungicide treated soils. Variation in lignocellulolytic populations among North American forests was observed and could explain differences in the rates of decomposition of substrates. Gene families encoding catabolic oxidative enzyme were expanded in shotgun metagenomes from DNA enriched with ^{13}C from lignin, confirming the importance of laccase, DyP-type peroxidases and vanillyl-alcohol oxidases for bacterial lignin degradation. We demonstrate the considerable potential of bacterial lignin degradation in soil, and identify a number of novel lignolytic taxa. The stable isotope probing method will be discussed in terms of limitations and strengths for studying soil communities. One noteworthy benefit of SIP was the vastly improved assembly of shotgun metagenomes. Overall, we conclude that bacterial decomposition is typified by a division of labour and that, in general, there is a greater diversity of cellulose-degrading taxa (a majority of which were previously characterized) compared to lignin-degrading taxa, most of which were not previously recognized.

AEM 125

Impact of repeated treatments of the Pesticides Chlorpyrifos or Tebuconazole on Soil Microorganisms using alternative methods

Veronika STORCK, Institut National de la Recherche Agronomique (INRA), Dijon, France, N ROUARD[1], J BÉGUET[1], D BRU[1], L BOTTERI[2], A SPOR[1], M DEVERS-LAMRANI[1], F MARTIN-LAURENT[1], [1]Institut National de la Recherche Agronomique (INRA), Dijon, France, [2]Aeiforia srl, Spinoff of Catholic University of the Sacred Heart, Fidenza, Italy

Pesticides can harm non-target organisms such as soil microorganisms involved in important ecosystem functions. During the pesticide authorization process of the European Union conducted by the European Food Safety Authority (EFSA), potential risks of pesticides on soil microorganisms are solely evaluated by estimating the impact on nitrogen mineralization which is a too general test to detect pesticide ecotoxicity towards microorganisms. In consequence, pesticide risk assessment conclusions on soil microorganisms edited by EFSA can differ from academic researches carried out with more advanced techniques allowing to monitor the abundance, diversity and activity of microbial communities. This is the case for the insecticide chlorpyrifos (CHL) and the fungicide tebuconazole (TCZ) evaluated for their ecotoxicological impact on soil microorganisms in this study using new experimental designs and advanced techniques. A new experimental design consisting in repeated exposure of soil microcosms to CHL or TCZ was used to evaluate the pesticide impact on (i) pesticide biodegradation, (ii) soil purification functions, and (iii) the diversity of soil bacteria. In response to repeated CHL treatments, the capability of soil microorganisms to degrade it increased. On the contrary, soil microorganisms kept a low ability to degrade TCZ. Soil purification functions were slightly (CHL) or not (TCZ) affected by pesticide treatments. However, the soil bacterial diversity changed in response to repeated exposure to both pesticides. *Nitrospira* (reducing effects) and *Lysobacter* (enhancing effects) were two of the genera that were modified in their abundance. This study provides new insights in the fate and ecotoxicological impact of CHL and TCZ on soil microorganisms.

AEM 105

The Tale of a Neglected Energy Source for Soil Microbial Communities

Sarah PICHÉ-CHOQUETTE, Armand-Frappier Institute - INRS, M. KHDHIRI[1], J. TREMBLAY[2], S.G. TRINGE[3], P. CONSTANT[1], [1]Armand-Frappier Institute - INRS, [2]National Research Council of Canada, [3]DOE - Joint Genome Institute

Several studies have demonstrated the fertilization effect of soil microbial communities in response to high concentrations of H_2 produced by nitrogen-fixing nodules, yet its cause has eluded us as of yet. Whilst it has been partly explained by the activity of a few H_2-oxidizing bacterial isolates, there is growing evidence that we are facing a much more complex phenomenon. Namely, we suggest that exposure to high concentrations of H_2 affects microbe-microbe interactions between H_2-oxidizing and non-H_2-oxidizing microorganisms, which in turn shapes microbial community structure and function. While most earlier work focussed solely on culture-dependent methods involving H_2-oxidizing bacteria, our study aims to decipher the effect of H_2 exposure on soil microbial community structure and function as a whole. In this light, our lab developed a dynamic microcosm chamber system that grants us the possibility to expose soil microcosms to precise H_2 mixing ratios. The ensuing incubations involved 2 distinct treatments in regard to our hypothesis: 0.5 ppmv H_2 as a control treatment simulating atmospheric H_2 permeating soils, and 10,000 ppmv H_2 as an elevated H_2treatment simulating H_2 hotspots found in soils, especially in the rhizosphere. The 15-day incubations make use of 3 different soil land-use types and have led to the collection and analysis of various biotic and abiotic data. Among those, both taxonomic and functional amplicon sequencing were performed, as well as metagenomic sequencing and qPCR assays. Measured microbial processes included trace gases uptake and production along with community-level substrate utilization profiles. Soil physicochemical parameters were also measured in order to characterize soil land-uses further. First off, microbial community profiles were distinct across land-use types and H_2 treatment. While we expected an increase in bacterial growth and overall metabolism due to an increase in available energy conferred by H_2, species richness changed significantly yet inconsistently between H_2 treatments whereas bacterial growth (assessed by qPCR) did not change in all land-uses. Moreover, this incoherent yet reproducible trend was also observed at the OTU (operational taxonomic unit) level as hundreds of bacterial and fungal OTUs were affected by the H_2 treatment. On the other hand, microbial functions had a common response to the treatment, albeit at varying intensity depicted by biotic and abiotic factors, as assessed by structural equation models. Notably, we observed an abatement in CH_4 uptake as well as a diversification of carbon substrate utilization potential, which implies that both specialist and generalist functions were affected by H_2 exposure. In sum, while these promising results show the potential of H_2 exposure on soil microbial communities, we argue that further examination is needed to truly grasp the H_2 fertilization effect and its underlying soil biogeochemical processes.

AEM 095

Adaptive Significance of Quorum Sensing-Dependent Regulation of Rhamnolipids by Integration of Growth Rate in *Burkholderia glumae*: A Trade-Off between Survival and Efficiency

Arvin NICKZAD, INRS-Institut Armand Frappier, E. DEZIEL[1], [1]INRS-Institut Armand Frappier

Quorum sensing (QS) is a cell density-dependent mechanism which enables a population of bacteria to coordinate cooperative behaviors in response to the accumulation of self-produced autoinducer signals in their local environment. An emerging framework is that the adaptive significance of QS in the regulation of production of costly extracellular metabolites (“public goods”) is to maintain the homeostasis of cooperation. We investigated this model using the phytopathogenic bacterium *Burkholderia glumae*, which we have

previously demonstrated uses QS to regulate the production of rhamnolipids, extracellular surface-active glycolipids promoting the social behavior called “swarming motility.” Using mass spectrometric quantification and chromosomal *lux*-based gene expression, we made the unexpected finding that when unrestricted nutrient resources are provided, production of rhamnolipids is carried out completely independently of QS regulation. This is a unique observation among known QS-controlled factors in bacteria. On the other hand, under nutrient-limited conditions, QS then becomes the main regulating mechanism, significantly enhancing the specific rhamnolipids yield. Accordingly, decreasing nutrient concentrations amplifies rhamnolipid biosynthesis gene expression, revealing a system where QS-dependent regulation is specifically triggered by the growth rate of the population, rather than by its cell density. Furthermore, a gradual increase in QS signal specific concentration upon decrease of specific growth rate suggests a reduction in quorum threshold, which reflects an increase in cellular demand for production of QS-dependent target gene product at low density populations. Integration of growth rate with QS as a decision-making mechanism for biosynthesis of costly metabolites, such as rhamnolipids, could serve to assess the demand and timing for expanding the carrying capacity of a population through spatial expansion mechanisms, such as swarming motility, thus promoting the chances of survival, even if the cell density might not be high enough for an otherwise efficient production of rhamnolipids. In conclusion, we propose that the adaptive significance of growth rate-dependent functionality of QS in biosynthesis of costly public goods lies within providing a regulatory mechanism for selecting the optimal trade-off between survival and efficiency.

SYMPOSIUM III: Microbial Systems Biology
Wednesday, June 21st, 2017
8:30 -10:00 AM
(MGCM)

MGCM 002

Systematic identification of a class of bacterial effectors with novel regulatory activities.

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Pathogens deliver complex arsenals of translocated effector proteins to host cells during infection, but the extent to which these proteins are regulated once inside the eukaryotic cell remains poorly defined. Amongst all bacterial pathogens, *Legionella pneumophila* maintains the largest known set of translocated substrates, delivering over 300 proteins to the host cell via its Type IVB, Icm/Dot translocation system. The sheer size of this arsenal raised the intriguing possibility that several of these proteins might functionally interact once inside the host. Backed by a few notable examples of effector-effector regulation in *L. pneumophila*, we sought to define the extent of this phenomenon through a systematic analysis of effector-effector functional interaction. To systematically map such interactions, we leveraged a champion of high-throughput biology. Using *Saccharomyces cerevisiae*, an established proxy for the eukaryotic host, we queried >108,000 pairwise genetic interactions between two compatible expression libraries of ~330 *L. pneumophila* translocated substrates. While capturing all known examples of effector-effector suppression, we identified several novel translocated substrates that suppress the activity of other bacterial effectors. In many, this regulation is direct - a hallmark of an emerging class of proteins called metaeffectors, or "effectors of effectors." Through detailed structural and functional analysis, we have shown that metaeffector activity derives from a diverse range of mechanisms, shapes evolution, and can be used to reveal important aspects of each cognate effector’s function. Metaeffectors, along with other, indirect, forms of effector-effector modulation, may be a common, unexplored feature of many intracellular pathogens.

MGCM 022

Sinorhizobium meliloti PHB cycle mutants display altered metabolite profile in nitrogen limited media

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Sinorhizobium meliloti stores excess carbon under unbalanced growth conditions as polyhydroxybutyrate (PHB). The PHB polymer is of considerable interest due to its potential role in initiation of nitrogen fixing symbiosis with *Medicago sativa* (alfalfa) and the development of commercial bioplastics. The nature of the regulation of PHB net accumulation has not yet been defined, although a recent RNAseq study has implicated Fnr transcriptional regulators. The effects of disruption of the PHB cycle on overall carbon cycling have not yet been elucidated. It was hypothesized that changes in carbon intermediate metabolism and carbon storage may be linked to transcriptional regulation. Null mutants in the PHB synthesis genes (*phbA, phbB, phbC*) and PHB degradation genes (*bdhA, phaZ* and *acsA2*) were used to examine changes in carbon cycling in the cell via extracellular NMR-based metabolomics in nitrogen limited growth conditions. Nineteen metabolites were detected in the media of one or more of the mutants. Of these nineteen metabolites, the concentrations of all but one were significantly different between the wild-type and at least one mutant. This demonstrates that disruption of the PHB cycle causes wide ranging effects on metabolite production and secretion in *S. meliloti* in nitrogen limited conditions. The PHB synthesis mutants showed a significant difference to the parental in most cases and the *bdhA* and *acsA2* mutants had differences for a few metabolites. The *phaZ* mutant and the glycogen synthase mutant, *glgA1*, always had a similar metabolite concentration to the parental. The lack of metabolite differentiation in the glycogen synthase mutant demonstrates that the observed metabolic changes are likely specific for PHB cycle disruption and not general carbon storage disruption. The metabolite findings were compared with previously generated RNASeq results for these mutants, to determine if there was evidence of transcriptional regulation of enzymes involved in the affected metabolic pathways. In the example of pyruvate, in which the *phbAB, phbB,* and *phbC* mutants had significantly more pyruvate accumulation than the parental strain, the genes associated with three enzymes involved in pyruvate metabolism were shown to be significantly differentially expressed in the PHB synthesis mutants (PEP carboxykinase, pyruvate dehydrogenase, and pyruvate orthophosphate dikinase). The genes encoding PEP carboxykinase and pyruvate dehydrogenase had increased transcript abundance, while the gene encoding pyruvate orthophosphate dikinase had decreased transcript abundance. These findings form the basis of *in vivo* support that links disruption of the PHB cycle to transcriptional regulation of enzymes involved in central metabolism. Follow up work examining activity of the associated enzymes will continue to delineate the physiological changes in metabolism in PHB cycle mutants.

MGCM 023

Comparative metabolic analysis of _Komagataeibacter spp._ grown in different carbon sources

Sierra DARGAN, University of Ontario Institute of Technology, A.J VARLEY[1], J.L STRAP[1], [1]University of Ontario Institute of Technology

Komagataeibacter are acetic acid bacteria regularly isolated from fruits and are model organisms for bacterial cellulose (BC) production. BC differs from plant cellulose in that it is free from impurities, making it an industrially relevant biopolymer. Applications of BC are diverse and include wound dressing, drug delivery systems and food products. While many studies in the literature attempt to improve BC production by growing *Komagataeibacter* in industrial waste water and fruit juices, there has been few attempts to systematically characterize the metabolic pathways that are involved in the production of BC using alternative carbon sources. This research compared the effect of carbon source on growth kinetics, acid

production, BC production and protein profiles of two species of *Komagataeibacter*: *K. xylinus* and *K. hansenii*. *K. xylinus* is a more abundant cellulose producer than *K. hansenii* and althoughboth belong to the same genus, they belong to different clades. Schramm-Hestrin (SH) medium was modified by substituting the canonical glucose (20 g/L) with 10 g/L and 15 g/L of glucose, 10 and 20 g/L of fructose and 20 g/L of sucrose. The wet weight, dry weight and thickness of BC was quantified while the BC crystallinity was analyzed by Fourier transform infrared spectroscopy. Surprisingly, glucose concentration was inversely correlated with both growth rate and final culture density. *K. xylinus*, but not *K. hansenii* displayed higher growth rate and final culture density when grown in fructose; however, this effect was not observed with *K. hansenii*. Both strains grew slowly when 20 g/L of sucrose was used as carbon source. The pH of glucose-grown cultures decreased from 4.3 to 3.8 after 48 hours of growth. Cultures grown in 10 g/L glucose significantly increased the pH of the medium to 5.1 by day 4, while cultures grown in 20 g/L glucose took 8 days to reach this pH. The pH of sucrose and fructose-grown cultures fluctuated between pH 5 to 5.5. Proteomic differences were observed between *K. xylinus* and K. *hansenii* grown in media of similar composition. This work represents an important first step in the characterization of the metabolic consequences of alternative carbon sources in BC production.

MGCM 027

Differential expression of non-coding RNAs by the pqs quorum sensing system in *Pseudomonas aeruginosa*

François D'HEYGERE, INRS-Institut Armand Frappier, S LE GUILLOUZER [1], Q LIOT[1], E DEZIEL[1], [1]INRS-Institut Armand Frappier

Bacterial inter-cellular communication, also called *quorum sensing*, is a process allowing coordination of gene expression at the level of a population, which is highly beneficial during the colonisation of various environment, like host infection. This process is based on the production of diffusible signal molecules able to stimulate the activity of cognate transcription factors after reaching a threshold concentration in the environment. In *Pseudomonas aeruginosa*, three different *quorum sensing* systems have been described to date. They regulate the large genomic repertory of this versatile bacterium containing several virulence or resistance factors, allowing this pathogen of global interest to colonise a wide range of hosts, including human. Less well understood than its two typical quorum sensing systems based on acyl-homoserine lactones as signaling molecules, the third system is mediated by molecules belonging to the 4-hydroxy-2-alkyquinoline (HAQ) family, especially PQS (*Pseudomonas* Quinolone Signal). Besides the function played by the various HAQs themselves, this system modulates the expression of various functions, among other production of phenazines such as pyocyanin, a virulence factor causing oxidative stress. While we have relatively good understanding of HAQ regulation and biosynthesis, the downstream regulatory function of this quorum sensing system on target functions is still poorly understood. Transmission of regulation on target genes such *phz* operons required for phenazines is based on the expression of *pqsE*, a gene belonging to the HAQ biosynthetic *pqs* operon. The expression of this operon, in turn, is induced by the transcription factor MvfR, which is activated by HAQ ligands such as PQS. Through its recently described thioesterase activity, PqsE is implicated in the synthesis of HAQs. However, recent studies showed that this activity is not required for its regulatory function on target genes. To better understand the regulatory function of PqsE, we have undertaken a genome-wide expression study using the RNA-seq method to acquire a comprehensive transcriptomic picture of genes, including small RNAs (sRNA), differentially regulated by *pqsE* expression. Data analysis revealed many interesting features. We show that *pqsE* expression impacts several antisense transcripts in some target genes of the *pqs* system. In addition, *pqsE* seems to regulate a few genes involved in c-di-GMP synthesis, an intracellular signaling molecule central in the planktonic-sessile lifestyles switch of this opportunistic pathogen. Finally, our data suggest that the 3' region right downstream of the *pqsE* gene seems to code for a sRNA, which we are currently characterizing. Preliminary investigations indicate that

these features play roles during establishment of the *pqs* system and expression of target genes. These finding will help better understand the functioning of the *pqs* quorum sensing system and the inter-cellular communication of *P. aeruginosa* during host infection.

MGCM 095

Studying the role of the AdeIJK RND efflux pump and its unlinked regulator AdeN in resistance and virulence in *Acinetobacter baumannii*

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Acinetobacter baumannii, a Gram-negative bacterium, is a problematic opportunistic pathogen due to its resistance to multiple antibiotics. Energy-dependent efflux of antibiotics mediated by proteins belonging to the Resistance-Nodulation-Division family is the predominant mechanism of intrinsic resistance in *A. baumannii*. AdeIJK is one such pump that is known to efflux a multitude to antibiotics in *A. baumannii*. AdeN, a TetR-family protein, has been previously shown to act as a repressor of the AdeIJK efflux pump. However, unlike other RND efflux pump regulators in *A. Baumannii* AdeN is not a linked regulator, it instead is located 813kbp upstream of the AdeIJK efflux pump operon. Previous study by our group of the Triclosan resistant mutant AB042, which has a 72bp deletion in *adeN*, showed significant changes in gene and protein expression, indicating that AdeN may be acting as a global transcription regulator in *A. baumannii*. To study the role of AdeN, we have created an unmarked deletion mutant of *adeN* (AB141) in ATCC17978. Quantitative reverse transcriptase PCR in combination with transcriptomic and proteomic analysis was performed on AB141 to determine if AdeN is acting as a global transcription regulator. Biofilm formation, motility, virulence, antibiotic susceptibility, and microbial growth were also evaluated to assess the phenotypic effect of *adeN* deletion on *A. baumannii*. Transcriptomic and proteomic analysis of AB141 showed that loss of *adeN* results in significant differential expression of 106 genes. Using a proteomics approach, we detected 31 proteins whose expression was altered. Phenotypic testing led to the observation that deletion of *adeN* results in decreased susceptibility to antibiotics and osmotic stress. The *adeN* deletion mutant also exhibited attenuated virulence, decreased biofilm formation and motility. In order to determine if the observed phenotypes were a result of overexpression of AdeIJK rather than loss of AdeN expression, an *adeIJK* deletion mutant (AB185) and a double mutant of *adeN* and *adeIJK* (AB186) were created and phenotypically evaluated. Intriguingly both *adeIJK* deletion mutants also exhibited attenuated virulence and motility compared to wild-type. These data indicate that AdeN and AdeIJK both are involved in the resistance and virulence of *A. baumannii*

SYMPOSIUM IV: Climate Change Microbiology
Wednesday, June 21st, 2017
10:30 AM - 12:00 PM
(AEM)

IS AEM 002

Climate change microbiology: insights from freshwater cyanobacteria

Kathryn COTTINGHAM, , C.C. CAREY[1], J.V. TROUT-HANEY[0], M.L. GREER[2], H.A. EWING[2], K.C. WEATHERS[3], [1]Virginia Tech, [2]Bates College, [3]Cary Institute of Ecosystem Studies

Climate change is a multi-faceted phenomenon with diverse impacts on ecological communities, including microbes. For example, the recent worldwide increase in cyanobacterial blooms in freshwater lakes is often attributed to warmer water temperatures. In this talk, I will explore cyanobacterial responses to climate change using case studies from deep temperate lakes and shallow Arctic ponds, with two main take-home messages. First, we cannot reliably predict these responses without considering the many different impacts of a changing climate on lakes, including altered stratification/mixing regimes and land-water connectivity alongside temperature. Second, we need to expand our conceptual models to include benthic cyanobacteria, including the benthic life stages of meroplanktonic taxa as well as those taxa found only in the benthos.
Case study 1: deep temperate lakes. Current forecasts for continued increases in cyanobacterial blooms in deep temperate lakes focus on the pelagic stage and ignore the fact that most bloom-forming taxa overwinter on or near the sediments, and thus must recruit from the sediments into the water column to initiate new blooms. In our long-term studies of the recruitment of benthic *Gloeotrichia echinulate* into the water column of Lake Sunapee (New Hampshire, USA), recruitment responds quite differently to stratification and mixing than water column abundances, suggesting we may need to take the complete life cycle of cyanobacteria into account to make reliable predictions. To explore the consequences of these differences - and whether this might be true for cyanobacteria more generally - we are using simulation modeling to evaluate cyanobacterial population dynamics across the complete life cycle in response to environmental conditions, including stratification and mixing, for *Gloeotrichia* and other species of cyanobacteria.
Case study 2: shallow Arctic lakes. High-latitude lakes and ponds are typically dominated by benthic primary producers, including colonial *Nostoc* and microbial mats, and thus have the potential to respond quite differently to a changing climate than phytoplankton-dominated systems. However, relatively little work has evaluated temporal trends in cyanobacteria or their toxins in these systems - even though the climate is warming much faster near the poles than in temperate regions. Our four-year study in shallow lakes in southwestern Greenland suggests that benthic taxa release cyanobacterial toxins into these systems, that toxin levels are increasing over time, and that the toxins are incorporated into both aquatic and terrestrial organisms in ways that demand further attention.

AEM 107

Microbial Community Development in Artificial Soil Columns as Affected by Water Table Fluctuations

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Water table fluctuations, which cause flooding and drying of soils, have been linked to enhanced degradation of soil organic carbon (SOC) and release of greenhouse gasses to the atmosphere. To improve our understanding of SOC degradation under changing moisture and oxygen dynamics, we carried out an automated soil column experiment with integrated monitoring of hydro-bio-geophysical processes under both

constant and oscillating water table conditions. An artificial soil mixture composed of quartz sand, montmorillonite, goethite and humus was used to provide a well-defined system. This material was inoculated with a microbial community extracted from a forested riparian zone. Microsensors, installed at different depths below the soil surface in the columns, recorded changes in redox potential and O_2 levels. The latter were measured continuously using high-resolution, luminescence-based *Multi Fiber Optode* (MuFO) microsensors. Effluxes of CO_2 and CH_4 were determined from headspace gas measurements. After 329 days, the columns were cut into 2 cm thick slices and analyzed for SOC, microbial biomass, and ATP content. Sequencing of the 16S rRNA genes targeted bacterial and archaeal communities with column depth at the final 329-day time point. Drying and wetting cycles affected CO_2 release from the soils significantly, with lower CO_2 fluxes during flooding periods and enhanced CO_2 fluxes after drainage compared to the CO_2 flux under constant water table. Furthermore, enhanced depletion of organic carbon was observed across the depth interval affected by the water table oscillations in the fluctuating water table column. This was associated with higher concentrations of ATP per mass of microbial biomass over the same depth interval. Furthermore, distinct microbial communities established between the unsaturated, saturated and transition zones in all columns.As expected, the transition zone was more broadly distributed in the fluctuating water table column. Automated functional assignments to 16S rRNA gene profiles implicated methanotrophy/methylotrophy by *Crenothrix* spp. as a dominant community function in the transition zone, bracketed by primarily aerobic and anaerobic processes in the oxic and anoxic zones, respectively. Overall, this experiment demonstrates the importance of local water table dynamics and redox conditions on SOC turnover and microbial community development.

AEM 134

Playing with Fire in the Alaskan Boreal Forest

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An increase in the number of wildfires has been attributed to changing climate regimes and associated alterations in precipitation patterns and temperatures. Although fire is a natural part of the boreal forests' disturbance regime, 30% of the largest fire years have been in the last decade resulting in generally larger burn areas. Thus we hypothesized that although fire likely had an immediate impact on soil communities, provided some organic layer remained, recovery would be seen within a few years. To test this hypothesis, we analyzed boreal forest soil overlying discontinuous permafrost near Fairbanks, Alaska. Fires in 2009 and 2015 resulted in an uneven burn with control sections exposed only to smoke. Perhaps not surprisingly, temperature, conductivity and pH were highest in the organic layers from the 2015 burn zone and these perturbations were correlated with the lowest soil respiration levels as measured by CO_2 emission. However, even 7 years after fire with now verdant overlying vegetation, soil pH was still elevated and respiration was depressed relative to control sites. DNA analysis as determined by rRNA Illumina MiSeq evaluation showed modest changes to the bacterial consortia. The most striking impact was seen in fungal populations with at least a 10-fold reduction in ectomycorrhizal tree symbionts (Agaricomyces) in the organic and mineral horizons of the sampled soils within both fire zones. As well, fatty acid methyl ester analysis confirmed the impact of the 2009 fire with an overall ~40% reduction in the recovery of total fatty acids, in addition to the specific reduction in fungal fatty acid signatures at both fire sites. Thus, even though the two burns were fragmented and not highly destructive, communities continued to be affected years after the event, prompting our suggestion that fires may have more lasting impact on the soil communities than we had previously appreciated. * This work was supported by an NSERC Discovery grant to VKW

AEM 104

Going beyond the surface: Microbial interactions in deeper horizons influence greenhouse gas emissions in a sandy Ontario agricultural soil

Lori PHILLIPS, Agriculture and Agri-Food Canada, B SEURADGE[1], D REYNOLDS[1], X YANG[1], C DRURY[1], [1]Agriculture and Agri-Food Canada

Microbial research in agricultural systems typically focuses on near-surface soil horizons (up to 30 cm in depth), where most microbial biomass is found. Agronomic management practices however, also alter edaphic and environmental conditions at depth, with probable impacts on the microbes that inhabit those deeper soils. We know very little about the intrinsic functional capacity of these microbial communities or how modifying soil conditions will affect that capacity. In this study we assessed how the removal of carbon-rich corn residues (stover), under consideration as a source of cellulosic ethanol for use in bio-fuels, impacted the nitrogen (N) cycling potential (mineralization, nitrification, and denitrification) of microbial communities to a depth of 1m. Soil cores were taken from a field trial in Harrow, Ontario that was designed to assess the impact of different stover removal rates (0, 25, 50, 75, 100%) on soil quality (carbon and N storage, soil physical-hydraulic properties) and greenhouse gas emissions (N_2O). Quantitative PCR and amplicon sequencing were used to measure functional and taxonomic responses (respectively). Any level of stover removal altered the capacity of the microbial community to cycle N, with the most pronounced impacts observed at depths greater than 30cm. For example, increasing rates of stover removal favoured microbes that perform incomplete denitrification (and produce N_2O) over those that perform complete denitrification (and produce N_2). Changes in these key microbial functional groups correlated with increased cumulative N_2O emissions. Understanding how the entire active soil microbiome responds to management will be a key factor in developing environmentally sustainable agro-ecosystems.

AEM 049

Emergence of cyanobacteria in a large macrophyte-dominated lake

Tyler HARROW-LYLE, University of Ontario Institute of Technology, A.E KIRKWOOD[1], [1]University of Ontario Institute of Technology

Lake Scugog is a large, marl lake in southern Ontario, and serves as a major headwater to the Trent-Severn waterway. Due to its shallow depth and large agricultural watershed, Lake Scugog is a nutrient-rich aquatic ecosystem that supports abundant plant and fish biomass. Commonly in macrophyte-dominated lake systems, phytoplankton abundance tends to be relatively low due to shading and competition for nutrients with macrophytes. This has generally been the case in Lake Scugog, where water chlorophyll a values (reflecting phytoplankton biomass) are typically low. In recent years, Lake Scugog has been experiencing changes in water quality and macrophyte abundance, including population collapses of the established non-native aquatic plant *Myriophyllum spicatum*, and the recent invasion of the non-native charophyte alga *Nitellopsis obtusa*. These observed changes raised concern about their potential impacts to the ecological integrity of the lake, particularly within the context of climate change. Thus to improve our understanding of ecosystem health in Lake Scugog, we collected baseline information on water quality and aquatic biota (macrophytes, phytoplankton and macroinvertebrates) across 12 sites in Lake Scugog from May to September, 2016. Over the course of the study, there were unusual occurrences of phytoplankton blooms at sites where they had not been reported before. Microscopic analyses revealed that these blooms were dominated by cyanobacteria, including the toxin-producing taxon *Microcystis*, and their abundance increased over the course of the sampling period. Statistical analysis indicated that cyanobacterial abundance did not correlate with the abundance of *N. obtusa*, but positively correlated with zebra mussel abundance. There was also a statistically significant positive relationship found between total phosphorus and cyanobacterial biomass. Although nitrogen-fixing taxa such as *Aphanizomenon* and *Anabaena* were common and had high numbers of

heterocysts (nitrogen-fixing cells), there was no relationship detected with total nitrogen concentrations in the lake. This is not entirely unexpected since total nitrogen is likely not a sensitive measure of nitrogen-limitation in the ecosystem. Overall, these initial observations in Lake Scugog suggest a potential regime shift may be occurring from macrophyte-dominated to phytoplankton-dominated. This would have detrimental ramifications for the prized sport fishery in the lake, and as such, warrants continued monitoring and study.

SYMPOSIUM V: Epidemiology in a Changing Pathogen Landscape
Wednesday, June 21st, 2017
10:30 AM - 12:00 PM
(I&I)

II 001

Enabling global genomic epidemiology - a key tool for public health and microbiology research

Fiona BRINKMAN, Simon Fraser University, Burnaby BC

Genomic epidemiology (combining microbial genomics data with epidemiological investigations to track the spread of infectious diseases) is transforming public health – and studies of pathogen evolution. As genome sequencing technology itself evolves, genomic epidemiology will become an increasingly useful tool for microbiologists. I will first review genomic epidemiology, its key uses, and some insights gained from it to date. Bioinformatics tools will be described that aim to facilitate easier genomic epidemiology analysis with more integrated, open data. However barriers remain regarding data sharing and data harmonization to permit more rapid, global analyses. This has become increasingly important in a world with expanding globalization of trade and travel networks – and associated spread of pathogens and antimicrobially resistant microorganisms. I will describe ways we can overcome these barriers, benefiting global public health – and microbiology research. I will also briefy describe recent large-scale analyses we've performed, providing further insight into potential global mobility of some key microbial genes of biomedical interest.

II 023

Experimental evolution of *Pseudomonas syringae* on a novel plant host

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*Pseudomonas syringae*is a diverse and globally significant pathogen that has adapted to cause disease in a wide range of plant hosts. These adaptations enhance host specific bacterial fitness, but also generate robust host-barriers that serve to limit the spread of individual *P. syringae* strains. Unfortunately, these host-barriers are not absolute, and many of the most devastating crop epidemics arise when pathogens evolve the ability to suppress or evade the immune response of a previously resistant host cultivar. How *P. syringae* strains and other plant pathogens overcome these robust host-barriers is poorly understood, so we sought to study a host-shift in real-time using the *in planta* experimental evolution of a bean pathogen, *P. syringae* 1448A, in the non-compatible host, *Arabidopsis thaliana*. Infiltration of *A. thaliana* Col-0 with *P. syringae* 1448A does not result in disease symptoms and realized *in planta* bacterial population sizes are nearly 1000-fold lower than those of *A. thaliana* pathogens. We find that serial passaging of *P. syringae* 1448A in *A. thaliana* results in an increased *in planta* growth rate, but their realized population sizes have yet to reach those of conventional *A. thaliana* pathogens. As *P. syringae* 1448A continues to adapt to its new host, we are using whole genome sequencing and functional assays to identify the molecular pathways that enable this strain to undergo a successful host-shift.

MGCM 057

Development of a Single Nucleotide Variant (SNV)-based subtyping scheme for the highly clonal Salmonella serovar Heidelberg

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Major enteric pathogens like Salmonella spp, which cause >5000 Salmonellosis cases annually in Canada, need to be promptly characterized to enable outbreak detection and source attribution. Due to the highly clonal nature of *Salmonella enterica* subsp. *enteric* serovar Heidelberg, current subtyping techniques are unable to distinguish related from unrelated isolates. Whole Genome Sequencing (WGS) is rapidly being adopted for routine surveillance in public health laboratories, due to the unprecedented amount of information that it provides. Current methods of WGS analysis produce phylogenetic trees that represent the relatedness of isolates but these are not readily interpretable by non-bioinformaticians without considerable experience. There is a need to codify WGS information into meaningful clusters, so that it can be communicated between people of diverse backgrounds within a public health framework. We have developed a reliable subtyping method for *Salmonella* Heidelberg using WGS data, and offer a simple nomenclature useful for outbreak detection and tracking, and for source attribution. The genomes of 531 Canadian and 1314 U.S. isolates of *Salmonella* Heidelberg from diverse sources were analysed using the SNVphyl pipeline developed at the Public Health Agency of Canada National Microbiology Laboratories (PHAC NML). Using strains with known epidemiological relationships, a panel of Single Nucleotide Variants (SNVs) was selected which passed rigorous discriminatory requirements. *Salmonella* Heidelberg was found to be extremely clonal, with an average of less than 10 SNP differences between unrelated clusters, indicating that cgMLST schemes will not provide sufficient resolution for subtyping. From our SNVphyl results, we found that*S.* Heidelberg is divided into 3 main lineages, which can be further divided into 51 distinct genotypes defined by 1 or more SNV positions. A hierarchical SNV-based nomenclature is proposed to group isolates together into related clusters. This subtyping scheme can be readily integrated into the Salmonella In Silico Typing Resource (SISTR) and into the Integrated Rapid Infectious Disease Analysis (IRIDA) platform, both developed at the PHAC NML, for *in silico* subtyping of isolates. The proposed SNV-based nomenclature, which can be applied to other clonal pathogens such as *S.* Enteritidis, can easily be communicated to relevant public health professionals for surveillance and outbreak analysis. This work will increase Canada's public health capacity to detect and respond to existing and emerging threats, and improve source attribution of clonal Salmonella serovars.

AEM 042

Identification of Food-Borne Pathogens Using Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF MS)

Anli GAO, University of Guelph, J. FISCHER-JENSSEN[1], C. COOPER[1], S. CHEN[1], D. SLAVIC[1], P. MARTOS[1], [1]University of Guelph

Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF MS) has been accepted for microbial diagnosis in clinical labs for its simple sample preparation procedure, quick turn-around-time, ease of use and low running cost for volume samples. Food-borne organisms were identified using a MALDI TOF MS instrument (VITEK, BioMerieux, QC) and compared with the results from biochemical and/or molecular techniques, in order to investigate the feasibility and reliability of MALDI TOF MS for use in a routine service lab for organism identification, pathogen screening, and potential stain differentiation and tracking. Fifty six organisms (ATCC strains and lab isolates) were tested in parallel with 16S rRNA gene sequence based PCR. MALDI TOF MS detected 52 of them correctly at genus level and 42

at species level with 2 wrong ID and 2 "No ID". The MALDI TOF results of 142 *Campylobacter jejuni* and 22 *C. coli* isolates are in agreement with the PCR results except for one isolate that was confirmed as *C. jejuni* by PCR but was identified as *C. coli* by MALDI TOF. Forty five out of 54 *Listeria* isolates were identified correctly to species. MALDI TOF MS was unable to distinguish some of the non-*L. monocytogenes* species. Of 345 *Salmonella* laboratory isolates taken directly from the working agar plates (BGS, MAC agar, XLT4) used in routine testing, 305 were correctly identified with 11 wrong ID and 24 "No ID". Correct identification rate was not different between BGS and MAC agar. Around 83% could still be identified correctly in the colonies of 31-61 days old, while 92% were correct at 3-10 days. Forty five suspect *Salmonella* negative by biochemical confirmation consisted mostly of *E. coli*, *Enterobacter* and *Cronobacter*, and 7 of them "No ID". Of the 143 lab isolates, mostly *Staphylococcus* (56), *Salmonella* (31) and *E. Coli* (17), tested by VITEK MS and BRUKER Biotyper in parallel, 127 (89%) agreed with each other at species level, 10 agreed only at genus level and 6 ended with different genus. MALDI TOF MS is a feasible tool for quick screening/identification. Although the results could be impacted by sample preparation and instrument set up parameters, well trained analysts and a stable performance instrument would solve most of the issues. Database updates with more spectra from new species should reduce the No ID rate. Update with spectra attained from colonies on working media should improve the identification rate and speed up the turn-around-time, especially in an out-break investigation. Application of MALDI TOF MS for strain differentiation, although has been reported with success in a number of species, is another great potential and is more challenging, which requires a) optimized sample preparation and MALDI TOF MS protocol, b) integration of data from different batch/instrument/time, c) strategy for supervised and unsupervised classification, and d) harmonization of qualitative and quantitative results before the data could be shared across labs.

II 017

Screen for Host Defence Peptide Resistance and Affiliated Mechanisms in Clinical Associates of Inflammatory Bowel Disease-associated *Escherichia coli*

Youn Hee CHO, Ryerson University, M.R. MICHAEL RENOUF[1], J.B.M. JOSEPH B. MCPHEE[1], [1]Ryerson University

The host microbiome is a known risk factor for the development of inflammatory bowel diseases (IBDs) but the mechanisms driving these alterations remain enigmatic. Host defense peptides (HDPs) are molecules secreted by the host that tightly regulate the composition of resident microbial communities. Several studies have found altered HDP expression profiles in patients with intestinal inflammation. Resistance to host defense peptides contributes to the success of at least one IBD-associated adherent-invasive *Escherichia coli* strain, NRG857c. We sought to investigate whether HDP resistance was conserved among clinical strains of IBD-associated *E. coli*. To do so, we screened a library of clinical *E. coli* isolates for resistance to two HDPs, LL-37 and hBD3. Our data shows that resistance to LL-37 was enriched in strains associated with ulcerative colitis (UC), while strains associated with Crohn's disease (CD) were better able to survive hBD3 treatment relative to treatment with LL-37. The upregulation of certain peptides has been shown to correlate to disease status in previous studies; for example, LL-37 expression is increased in the gut of UC patients. The increased concentration of specific peptides in different disease states is a possible selection pressure conferring high fitness to only those strains able to resist killing via these antimicrobials. This may be a mechanism by which these strains are able to thrive in these otherwise hostile conditions, explaining their enrichment in certain disease states. We also performed a molecular screen of these clinical isolates for a variety of known genetic determinants of peptide resistance. The presence of these alleles alone was not always indicative of increased peptide resistance, suggesting that the regulation of these genes is crucial for the overall phenotype. For example, an *ompT* promoter which has been shown to have increased transcriptional activity was more commonly seen in UC isolates, which were also associated with LL-37

resistance. Some of the strains in the library were highly resistant to one or both peptides tested, yet carried none of the peptide resistance alleles screened for. These strains are of great interest for further study, as they may reveal novel mechanisms of HDP resistance.

SYMPOSIUM VI: Structural Biology
Wednesday, June 21st, 2017
10:30 AM - 12:00 PM
(MGCM)

MGCM 001

Discovery of a novel flagellin family in diverse bacteria that forms enzymatically active flagella

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Bacterial flagella are cell locomotion organelles composed primarily of the polymeric protein flagellin. Although flagellins are known to play a role in flagellar assembly and host adhesion, no flagellins to date have been identified with enzymatic function. Through a bioinformatic survey of over 150,000 flagellin sequences from genomic databases, we identified and characterized the first family of enzymatic flagellins. These flagellins have acquired a metalloprotease domain within their central hypervariable region, are present in the genomes of over 100 phylogenetically diverse species. In the animal pathogen *Clostridium haemolyticum*, proteolytic flagellins formed the second most abundant component of purified flagella, and the protease domain was shown by immunoelectron microscopy to localize to the surface of extracellular flagellar filaments. Intact flagella were proteolytically active and cleaved hundreds of peptides in a high-throughput assay, which indicated a specificity toward extracellular matrix proteins. Our studies therefore reveal a novel component of bacterial flagella and a new role for flagella as enzymatic biopolymers. Flagellum-mediated extracellular proteolysis expands our understanding of the functional plasticity of this important bacterial organelle, and may impact several physiological functions from biofilm formation to virulence.

MGCM 091

Phage moron JBD26-61 promotes resistance to phage infection by disrupting cyclic di-GMP mediated type-iv pilus biosynthesis

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Bacteriophages (phages) follow two distinct life cycles, the lytic cycle resulting in phage particle production and bacterial cell death, and the lysogenic cycle wherein the viral genome is integrated within the bacterial chromosome as a prophage. The presence of prophages within bacterial genomes has been associated with increased virulence and survivability within human hosts through expression of “morons”, a group of genes not directly involved in the lytic or lysogen pathways. A systematic study by our group utilizing a library of phages (JBD) specific to the opportunistic pathogen *Pseudomonas aeruginosa* characterized phenotypic changes associated with prophage expression including decreased twitching motility, biofilm production and

phage resistance. By comparing the genomes of these phages, non-conserved morons were identified and subsequently expressed in *P. aeruginosa* to identify their role in host modification. Expression of moron JBD26-61 resulted in resistance to phages targeting the type-IV pilus (T4P) in both *P. aeruginosa* PAO1 and PA14. In addition, expression of JBD26-61 attenuated twitching motility in three separate *P. aeruginosa* strains: PAO1, PA14 and PAK, demonstrating a broad-range of activity against the T4P machinery. This makes JBD26-61 an attractive target for downstream investigations of phage-mediated T4P resistance mechanisms. The crystal structure of JBD26-61 revealed a dimeric four-helix bundle with an electronegative surface. To identify the host-protein target of JBD26-61, the bacterial two-hybrid system was utilized to screen for protein-protein interactions. Co-expression of JBD26-61 and PilZ produced strong red pigment and high β-galactosidase activity indicative of an interaction. PilZ is involved in c-di-GMP signalling of T4P biosynthesis by interacting with FimX, a phosphodiesterase, and the ATPase extension motor PilB. A Δ*pilZ* knockout is completely resistant to phage infection supporting its identification as a target for phage inhibition. No strong interactions between JBD26-61 and other members of the PilZ family were detected demonstrating the specificity of JDD26-61 to the T4P. We predict JBD26-61 prevents PilZ from binding to PilB thereby disrupting c- di-GMP signalling. Site-directed mutagenesis of PilZ residues involved in PilB interaction will be screened for loss of β-galactosidase activity when co-expressed with either JBD26-61 or PilB demonstrating that JBD26-61 binds to the same interface as PilB. To our knowledge this is the first phage gene affecting a c-di-GMP signalling pathway to promote phage resistance.

AEM 114

Bacterial tyrosine phosphorylation of a multicargo chaperone regulates hierarchical type III effector secretion and supports enteric disease

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Enterohemorrhagic and Enteropathogenic *E. coli* (EHEC and EPEC) are global enteric pathogens that causes serious gastrointestinal disease and inflict a significant burden on healthcare systems worldwide. The *E. coli* type III secretion system (T3SS) is a needle-like complex that mediates rapid and direct injection of multiple effector proteins into host cells to subvert normal signalling pathways and promote bacterial colonization. Like many Gram-negative pathogens, *E. coli* must tightly regulate T3SS activity, which at one level involves a multitude of bacterial chaperone proteins in the cytosol that bind to and stabilize effectors prior to their injection into a target cell. A recent phosphotyrosine proteome study of enterohemorrhagic *E. coli* (EHEC) O157:H7 and *E. coli* K12 identified an unprecedented abundance of tyrosine-phosphorylated proteins. The extent of this reversible post-translational modification was previously thought to be limited among bacteria, yet was discovered to occur among proteins involved in type III secretion-mediated virulence. Specifically, two consecutive tyrosine residues, Y152 and Y153, located within the unique C-terminal domain of the type III secretion chaperone (T3SC) CesT were identified as sites of phosphorylation. We set out to determine the impact of CesT phosphorylation on type III secretion-mediated virulence of EPEC, which critically requires CesT to promote efficient secretion of multiple effector proteins. *E. coli* strains deficient for CesT phosphorylation were generated with recombinant DNA mutagenesis techniques on the *cesT* locus. These CesT sequence variants express specific tyrosine (Y) to phenylalanine (F) substitutions, a strategy that retains protein structure but prevents phosphorylation due to absence of a critical oxygen atom on F residues. With well-established infection assays we have characterized the significance of CesT phosphosite mutations on effector translocation and infection progression. Positional Y to F mutations result in unique changes to

secretion of CesT-dependent type III effectors, and loss of phenotypes associated with disease progression *in vitro.* These observations were validated *in vivo* using the closely related mouse pathogen *Citrobacter rodentium,* which requires CesT, and specifically CesT phosphosites for significant intestinal colonization. This study is the first to demonstrate a functional relevance of site-specific bacterial tyrosine phosphorylation for type-III secretion chaperone protein trafficking events. Given the requirement for CesT phosphorylation in a natural infection model, these findings introduce an unexplored potential for currently unknown bacterial kinase inhibitors to help combat EPEC and EHEC infections.

MGCM 043

Deciphering the Biosynthetic Logic of Bacterial Cell Surface Phosphonate Tailoring: Towards Pathway-Specific Inhibitors

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Phosphonates represent an overlooked functional group in biology, and despite the success of phosphonate natural products like fosfomycin we know almost nothing about phosphonate biology. For example, phosphonates are predicted to widely decorate bacterial surfaces, yet little attention has been paid to their structure, function, taxonomic distribution or biosynthetic origin. As reduced versions of phosphate possessing a stable C-P bond, phosphonates may confer a selective advantage in anaerobic environments or resist degradation by lysosomal enzymes. To this end we have: (i) detected C-P bonds in diverse bacterial phyla, and (ii) discovered a phosphonate-specific cytidylyltransferase enzyme that we have named PngC. Interestingly, PngC is related to the phosphocholine (ChoP)-specific cytidylyltransferase LicC, which is known to contribute to *Streptococcus pneumoniae* virulence in a mouse model. Similarities among *png* and *lic* gene clusters suggest that cell surface installation of both ChoP and phosphonates like 2-aminoethylphosphonate (AEP) employ similar biosynthetic logic. Significantly, we have determined that LicC from *S. pneumonia* is highly specific for ChoP (but not AEP), while PngC from the human oral microbe *Atopobium rimae* is highly specific for AEP (but not ChoP). This divergent substrate specificity implies that pathway-specific inhibitors can be developed for (i) probing the biological roles of each pathway, and (ii) narrow-spectrum therapeutic intervention to selectively disrupt *S. pneumonia* virulence while minimizing collateral damage to *pngC*-containing microbiota.

MGCM 089

Characterization of FHA Domains Involved in *Saccharomyces Cerevisiae* dNTP Regulation and Double-Strand Break Repair

Geburah STRAKER, University of Waterloo, BP DUNCKER[1], [1]University of Waterloo

Over 2000 Forkhead-associated (FHA) domain-containing proteins have been identified to date in both eukaryotic and prokaryotic organisms including kinases, phosphatases and transcription factors. Initially characterized as the only known protein-protein interaction motif with phosphothreonine residue binding specificity, recent evidence from a study by the Duncker Lab that characterized the minimal interacting surfaces between the Rad53 FHA1 domain and the H-BRCT domain of Dbf4 in the model organism *Saccharomyces cerevisiae* suggested the existence of non-canonical binding surfaces on at least some FHA domains that do not rely on the phosphothreonine-binding patch. The present research aims to study the prevalence of protein-protein interactions in *S. cerevisiae* that operate using this novel non-canonical lateral

surface patch of FHA domains and their functional significance in cell growth and survival mechanisms in response to genotoxic stress. In order to investigate the existence and importance of further non-canonical FHA protein interaction domains, bioinformatics analysis was used to identify candidate conserved surface patches, site-directed mutagenesis was used to alter key amino acids and yeast two-hybrid analyses were used to compare physical interactions between wild type and mutant FHA domains with ligands involved in the dNTP regulation and double-strand break repair pathways. Initial analysis of the interaction between the FHA domain of DNA damage UNinducible (Dun1), a budding yeast cell cycle checkpoint kinase involved in regulating dNTP synthesis, and Damage-regulated Import Facilitator (Dif1), a nuclear importer of the two small subunits of ribonucleotide reductase, has shown that the canonical phosphothreonine binding patch is essential to the establishment of the interaction. Site-directed mutagenesis of four conserved residues within the lateral surface patch of the Dun1 FHA domain have been generated to mutate the lateral surface patch. Yeast two-hybrid analysis to determine if the mutation of the lateral surface patch is also sufficient to disrupt the interaction between the Dun1 FHA domain and Dif1 is underway. In terms of double-strand break repair, the MEiotic Kinase (Mek1) - REDuctional division (Red1) - HOmolog Pairing (Hop1) mechanism for regulating the choice between intersister-chromatid and interhomolog repair of double-strand breaks is also being investigated with Mek1 as the FHA domain-containing protein. Preliminary yeast two-hybrid data has confirmed a physical interaction between the two synaptonemal complex proteins, Red1 and Hop1. Yeast two-hybrid assays are underway in an attempt to detect a physical interaction between the Mek1 FHA domain and Hop1 and/or Red1, and recent bioinformatics analysis has identified a candidate conserved lateral surface patch of the Mek1 FHA domain.

SYMPOSIUM VII: Computational Methods in Microbiology
Thursday, June 22nd, 2017
8:30 - 10:00 AM
(Unified Theme)

IS 003

Enterotypes, Autism, Aliens and the Elderly: the microbiome as a composition

Greg GLOOR, University of Western Ontario, London ON

Datasets collected by high throughput sequencing (HTS) from 16S rRNA gene sequencing or from metagenomic sequencing or from meta-transcriptomics, are commonplace and being used to study human disease states, ecological differences between sites, and the built environment. There is increasing awareness that microbiome datasets generated by (HTS) are compositional because reads for a given sample have an irrelevant (or constant) sum. Many investigators are unaware of this, or treat their data as conditionally impositional, or make specific assumptions about the properties of the data. I will alert investigators to the dangers inherent in these approaches, and point out that HTS datasets derived from microbiome studies can and should be treated as compositions at all stages of analysis. I will illustrate the pathologies that occur when compositional data are analyzed inappropriately, introduce the nature of compositional data, and finally give a use example from a 1000 sample cross-sectional cohort on how compositional data analysis can be adapted to microbiome datasets.

AEM 093

Data-Driven Discovery of Small Molecules from the Human Microbiota

Walaa MOUSA, McMaster University, NA MAGARVEY[1], RZ ZVANYCH[1], DL LIU[1], NM MERWIN[1], BA ATHAR[1], MS SKINNIDER[1], MC CANNON[1], CD DEJOING[1], MR RANIERI[1], XL LI[1], [1]McMaster University

Decades of research on human microbiota have revealed much of their structural diversity and their direct link to health and disease. However natural products secreted by our microbial partners and their effect on our biology are still largely unknown. Microbiota-derived molecules are thought to be co-evolved with selectivity towards human cellular targets and hence represent novel therapeutics. The expanding pool of genomic information has led to development of several bioinformatics tools that aim to identify biosynthetic clusters and predict their encoded small molecules. However, connecting these predictions to actual metabolome data is still lagging behind. We sought to bridge this existing gap by developing a bioinformatics platform capable of connecting the predictive power of our in-house developed PRISM with the actual mass spectrometry files to assist with the discovery of novel natural products. This led to the development of ComputationaL Analyses of Mass Spectra (CLAMs).One of the most recently developed software suites, PRISM, allows for entire genome analysis with complete annotation of domain organization, including automatic prediction of the chemical structures of final products based on the monomer selectivity. These tools, for the first time, have connected genomic prediction to real metabolomics data and led to cataloguing of the microbiota exclusive chemistry. As a validation of the concept, two of these microbiome exclusive molecules have been targeted for further purification and downstream structural elucidation and activity profiling. Two novel small molecules have been characterized from clinical isolate of *Pseudomonas aeruginosa* and *Faecalibacterium prausnitzii*. Preliminary data suggest their potential as immune modulatory agents. Therefore, we see CLAMS as effective tool for small molecule discovery that can be applied towards

further metabolomics de-orphaning of the human microbiomewhich will revolutionize the field of natural products discovery.

MGCM 048

Predictive modelling of a batch filter mating process

Brian INGALLS, University of Waterloo, A MALWADE[1], A NGUYEN[1], P SADAT-MOUSAVI[1],[1]University of Waterloo, Waterloo ON

Quantitative characterizations of horizontal gene transfer mechanisms are needed for understanding and predicting the dynamics of gene distribution in natural and engineered systems. In this study, we developed a mathematical characterization of plasmid conjugation between two bacterial populations (filter mating). We mated two *E.coli* strains. The donors harboured the self conjugative, GFP-coding plasmid pKJK10. The recipients expressed RFP from the plasmid pSB1C3. Time series assays were made by flow cytometry to quantify the distribution of the three subpopulations involved in the filter mating process (GFP+/RFP- donors, GFP-/RFP+ recipients, and GFP+/RFP+ transconjugants). Corresponding measures of optical density determined the temporal variation in the abundance of each population. We used the data to fit ordinary differential equation models of the process. Model comparison tools were applied to arrive at an optimal model formulation, and the accuracy of the best-fit parameter estimates was assessed via uncertainty analysis. We tested the model's predictive power by comparing model simulation to experimental results that demanded extrapolation from the training data. These comparisons provide evidence that the model can be successfully used as a predictive tool for characterizing horizontal gene transfer mechanisms in natural or synthetic systems.

AEM 043

Phage Term: A Fast and User-friendly Software to Determine Bacteriophage Termini and Packaging Mode Using Randomly Fragmented NGS Data

Julian GARNEAU, Université de Sherbrooke, F. DEPARDIEU[1], L.C. FORTIER[2], D. BIKARD[1], M. MONOT[1], [1]Institut Pasteur de Paris, [2]Université de Sherbrooke

Bacteriophages produce virion particles protecting their nucleic acid content, mainly as linear dsDNA form. In this study we investigate how the information gathered by high throughput sequencing technologies can be used to determine the DNA termini and packaging mechanisms of dsDNA phages. The wet-lab procedures traditionally used for this purpose rely on the identification and cloning of restriction fragment which can be delicate and cumbersome. Here, we developed a theoretical and statistical framework to analyze DNA termini and phage packaging mechanisms using next-generation sequencing data. A software, PhageTerm, was validated on a set of phages with well-established packaging mechanisms representative of the termini diversity: 5′cos (lambda), 3′cos (Efm1), pac (P1), headful without a pac site (T4), DTR (T7) and host fragment (Mu). In addition, we applied our methods to decipher the termini of 9 Clostridium difficile phages and 5 phages whose sequences where retrieved from the sequence read archive (SRA). Our methods are implemented in the PhageTerm software which we are making freely available. For direct graphical interface operation, a Galaxy wrapper version is also available at https://galaxy.pasteur.fr.

AEM 012

Amplytica: Bringing Microbial Ecology to the Cloud.

Lee BERGSTRAND, Thompson Rivers University, M. J. MCINNES[1], J. D. VAN HAMME[1], [1]Thompson Rivers University

The Amplytica Cloud Platform (ACP) is a software system for building large scale bioinformatics applications on commercial cloud computing infrastructure with a focus on microbial ecology workloads. ACP makes use of emerging open source cloud technologies such as Docker, Salt-Cloud and RabbitMQ, Binary Large Object (BLOB) stores such as Amazon S3, and cloud databases such as Heroku PostgreSQL. The platform is designed to be distributed across servers which do not need to be in a cluster or even on the same cloud provider. Components can be hosted on-site to utilize existing hardware and allow for greater data security. Components are stateless meaning that no sequence data is stored inside them allowing for failure with minimal data loss. Since the system is distributed, components can be turned on and off on demand allowing end users to only pay for individual bioinformatics processing jobs rather than for longterm servers. This allows for scalability and at least 60% cost savings over traditional bioinformatics architectures. In a microbial ecology context, ACP processing components wrap the QIIME microbial ecology pipeline allowing it to cluster OTUs in a closed, or in the future, open-reference guided fashion on high-RAM cloud virtual machines. The platform is targeted towards high throughput applications such as bioreactor monitoring and optimization, personalized medicine and sequencing centres with the ability to process, store and organize hundreds of samples per month. The platform includes facilities for sequence quality filtering and quality control, compressed storage of sequence data, sample and project management, and prototyped functionality for metadata capture and integration. Unlike competing platforms, ACP can be hosted on public or private clouds by Canadian cloud providers and will support multiple sequencer vendors in the near future.

SYMPOSIUM VIII: Microbial Arms Race: Antibiotics and Resistance
Thursday, June 22nd, 2017
8:30 - 10:00 AM
(I & I)

IS II 003

A Thermosensory Diguanylate Cyclase that Mediates Temperature-Dependent Bacterial Biofilm Development

Joe HARRISON, University of Calgary, Calgary AB

Many bacteria utilize the intracellular second messenger cyclic diguanylate (c-di-GMP) to control virulence, motility, and extracellular polymer production. Although a growing body of work has elucidated the biochemistry of c-di-GMP synthesis, degradation and effector function, very little is known about how external stimuli are perceived by c-di-GMP regulatory networks. Here we report the discovery of c-di-GMP signaling proteins that function as biological thermostats. The archetype of these proteins is the thermosensing diguanylate cyclase (TdcA) from the opportunistic pathogen *Pseudomonas aeruginosa*. TdcA synthesizes c-di-GMP and controls biofilm formation in response to body temperature. Site-directed mutation and domain-swapping analyses indicate that heat-sensing is mediated by a cofactorless Per-Arnt-SIM (PAS) domain, which is a previously undescribed function for this widespread family of protein domains. Purified recombinant TdcA displays "thermostatted" enzyme kinetics: it is thermally activated above a threshold temperature (27 °C), and displays a reaction rate that linearly increases more than 50-fold over a 15 °C range. These heat-dependent enzyme kinetics, which cannot be approximated by known biophysical models describing Q_{10} temperature coefficients, enable rapid physiological change over narrow temperature ranges. Using intravital imaging, we demonstrate that *tdcA*$^+$ *P. aeruginosa* suppresses early innate immunity in the murine lung, and that immune evasion depends on the c-di-GMP-regulated extracellular polysaccharides PEL and PSL. TdcA orthologues are widespread in Proteobacteria, and putative heat-sensing PAS domains are linked with hundreds of predicted diguanylate cyclases and c-di-GMP-specific phosphodiesterases in the PFAM database. Our data suggest that heat-sensing is a widespread function of c-di-GMP networks, and that this function enables bacteria to build biofilms in habitats with desirable temperatures - including the mammalian body.

MGCM 068

Does an Epidemic and Transmissible Cystic Fibrosis Strain of *Pseudomonas aeruginosa* Employ the Type Six Secretion System in Strain Replacement?

Jenny NGUYEN, University of Calgary, J. DUONG[1], M.D. PARKINS[1], D.G. STOREY[1], [1]University of Calgary, Calgary AB

Pseudomonas aeruginosa is an opportunistic bacterial pathogen that colonizes cystic fibrosis (CF) lungs and has been associated with chronic infections and accelerated clinical decline. Previously it was thought that CF patients were colonized with unique (non-clonal) *P. aeruginosa* isolates in their lungs. While the majority of patients harbor local unique isolates, clonal epidemic strains of *P. aeruginosa* have been documented in multiple clinics worldwide. An epidemic strain called the Prairie Epidemic Strain (PES) has been identified in one-third of patients at the Calgary Adult CF Clinic. It is believed that PES has been present in our patient cohort since 1987. Recently, PES has been found to replace other unique non-epidemic strains of *P. aeruginosa* in the CF lungs, leading to the investigation of the mechanisms in which PES achieves strain replacement. We specifically focused on the type six secretion system (T6SS), a transmembrane nanoweapon that injects lethal effectors into targeted cells. Whole genome sequencing of a PES isolate indicated that the

full set of genes required to synthesize the T6SS was present. Therefore, we hypothesized that PES utilizes the T6SS to competitively kill other *P. aeruginosa* isolates in the lung to displace them. *In vitro* cell-to-cell killing assays were performed to observe the competitive ability of a PES isolate against other bacterial species in order to determine whether the T6SS was active. Moreover, the competitive ability of PES was assessed against the lab strain PAO1 and a non-epidemic unique *P. aeruginosa* isolate. Viable colony counts were performed to determine which isolate was dominant and better able to compete. We found that PES competes similarly to PAO1 in that both isolates were able to kill competing bacteria, which suggests that the T6SS may be functional in PES. However, PES was unable to kill the unique non-epidemic CF isolate. An *in vivo Drosophila melanogaster* (fruit fly) infection model was also used to investigate the role of the T6SS in strain replacement. Flies were initially challenged with the unique isolate and then after 3, 6 or 9 days, the flies were coinfected with PES. Fly survival was monitored daily over 14 days and colony counts were determined three days after the flies were transferred to vials containing PES. These assays did not coincide with the cell-to-cell killing assays since PES was the dominant isolate *in vivo* and was able to compete with the non-epidemic CF isolate in a fly model. Furthermore, the sooner the flies were coinfected with PES, the better the epidemic strain was able to compete against the non-epidemic isolate. These results demonstrate that the competitive ability of PES against the unique non-epidemic CF isolate differs *in vitro* as opposed to *in vivo*. Furthermore, findings suggest that PES may employ the T6SS to outcompete the displaced unique isolate but other factors may also be involved in strain replacement in the CF lung environment.

II 033

Functional relationships of RND efflux pumps in *Staphylococcus aureus*

Jamie HALUCHA, University of Western Ontario, H. ALNARSERI[1], M.J. MCGAVIN[1], [1]University of Western Ontario, London ON

Staphylococcus aureus is a Gram-positive opportunistic pathogen well known to colonize and infect human skin and soft tissue. The emergence of methicillin resistant *S. aureus* (MRSA) has become a significant disease burden worldwide, and *S. aureus* USA300 is a prominent strain of community-associated MRSA responsible for severe invasive infections in humans. However, to colonize successfully and establish infection, *S. aureus* must overcome host defense mechanisms, including antimicrobial unsaturated free fatty acids that are encountered on the skin and in soft tissue abscesses. A contributing factor known to play a vital role in the intrinsic defense mechanisms of bacterial pathogens is the resistance-nodulation-division (RND) superfamily of efflux pumps, which are ubiquitous among bacteria. The staphylococci have two genes encoding RND efflux pumps; *farE*, which promotes efflux of antimicrobial fatty acids, and an uncharacterized gene we have named *femT*. Although RND pumps are known to play critical roles in physiological function and antimicrobial resistance, the function of FemT, and the relationship between FemT and FarE in staphylococci have not been identified. We hypothesize that both FarE and FemT support critical physiological processes that are defining traits of the staphylococci. Since *femT* is co-transcribed with *femX*, a gene essential for peptidoglycan synthesis, we hypothesize that FemT plays a role in cell wall synthesis by promoting transport of a peptidoglycan precursor. We further hypothesize that functional redundancy may occur between FemT and FarE, as both are implicated to play a role in lipid transport. Using established assays, we have tested the phenotype of a *S. aureus*^{femT} deletion mutant under conditions that reduce cross-bridge formation. Here, we show that mutants defective in FemT are mildly more susceptible to vancomycin, and grow faster than wildtype *S. aureus* in Mueller-Hinton broth. Most notably, when evaluating the relationship between these transporters using the luciferase reporter assay, FarE expression in linoleic acid was abolished in mutants deficient in FemT function. These findings suggest an interplay between the two RND transporters, and cumulatively, represent the first description of both RND efflux systems operating in *S. aureus*. Detailed evaluation of RND transporter function will provide novel insight

into the biology of staphylococci and further our understanding of this ubiquitous family of proteins in Gram positive pathogens.

II 014

Functional genomic analysis of echinocandin resistance in *Candida albicans*

Tavia CAPLAN, University of Toronto, EJ POLVI[1], N ROBBINS[1], LE COWEN[1], [1]University of Toronto, Toronto ON

Candida albicans is a leading cause of fungal infections, with mortality rates of ~40%. Effective treatment is hindered by the paucity of antifungal drugs, as well as the ability of fungal pathogens to exploit diverse strategies to tolerate and develop resistance to antifungals. Defining the cellular circuitry governing drug tolerance and resistance is key to identifying novel strategies to block resistance and enhance drug efficacy. To identify novel mechanisms of tolerance to the newest class of antifungals, the echinocandins, we screened two *C. albicans* mutant libraries collectively covering ~50% of the genome. We identified 13 novel regulators of echinocandin tolerance, most of which were specifically required for cell wall stress responses. Four genes, *TSC11*, *KEX2*, *RPS5* and *CCT8*, also enabled clinically-relevantechinocandin resistance. Cct8 is one of eight subunits of the chaperonin containing TCP-1 (CCT) complex, required for the folding of proteins such as actin. By depleting each CCT subunit, we implicated the entire CCT complex in echinocandin tolerance. Chemical inhibition of actin phenocopied depletion of the CCT complex, suggesting that this complex mediates echinocandin tolerance at least in part through actin. Analogous to actin perturbation, depletion of *CCT8* activated the cell wall stress response, as demonstrated by activation of the mitogen-activated protein kinase Mkc1. This work uncovers novel regulators of echinocandin tolerance and resistance, and unveils a role for the CCT complex in echinocandin resistance.

II 083

Discovery of an antibiotic effective against the opportunistic pathogen, *Acinetobacter baumannii*

Ashley WILLIAMS, University of Regina, J. STAVRINIDES[1], [1]University of Regina, Regina SK

Multi-drug resistant human pathogens are a serious worldwide healthcare concern, spurring the need to develop new therapeutics to address evolving bacterial diseases. *Acinetobacter baumannii* is one such pathogen deemed a critical threat in February 2017 by the World Health Organization, along with *Pseudomonas aeruginosa* and members of *Enterobacteriaceae.* Many isolates of *A. baumannii* are resistant to one or more last-line antibiotics, and a few are resistant to all available treatment options. Infections by *A. baumannii* readily spread within hospital settings especially in areas of war and natural disaster, targeting primarily wounded, critically ill, or debilitated individuals. The objective of this research is to identify and characterize antibiotics that are effective against *A. baumannii*. Members of the enterobacterial group, *Pantoea*, produce a variety of compounds with antimicrobial activity. To determine if *Pantoea* produce an antibiotic effective to treat *A. baumannii*, a collection of over 100 *Pantoea* isolates was surveyed using an agar overlay assay. Two antibiotic-producing *P. agglomerans* strains were identified, and the genes responsible for antibiotic production were determined by screening a library of transposon mutants. Interrupted genes of mutants no longer producing the antibiotic were amplified with inverse PCR, sequenced, and identified. Genetic comparisons revealed the same cluster of antibiotic genes in both *P. agglomerans* strains, suggesting that the same antibiotic is produced by both bacteria. A preliminary survey of the spectrum of activity revealed the antibiotic to have activity against a variety of Gram-negative pathogens, including *Pseudomonas aeruginosa* and several members of *Enterobacteriaceae*. Future work includes the chemical extraction and spectral analysis of the antibiotic to determine the structure and identity. In an age of antibiotic resistance, the identification of new antibiotics and antibiotic sources will continue to be of critical importance.

SYMPOSIUM IX: Microbial Origins and Symbioses
Thursday, June 22nd, 2017
8:30 - 10:00 AM
(Unified Theme)

IS 001

One plus one equals one: historical and modern perspectives on symbiosis

John ARCHIBALD, Dalhousie University, Halifax, NS

The evolution of the eukaryotic cell is a puzzle that has challenged biologists for over a century. The prokaryote-eukaryote cellular divide is enormous and many fundamental questions about the origin of eukaryotes and their endosymbiotically derived mitochondria and plastids remain unanswered. Here I will place the present-day study of cellular evolution in a historical context, highlighting advances and lingering uncertainties in our understanding of the role of endosymbiosis in the evolution of eukaryotic organelles.

II 030

Rapid Experimental Evolution of Antibiotic Resistance

Brandon FINDLAY, Concordia University, NG GHADDAR[1], [1]Concordia University, Montreal QC

Once hailed as wonder drugs, many of our safest and strongest antibiotics have been rendered obsolete by the steady increase in antibiotic resistance. This culminated last year in a fatal infection in Nevada, involving a strain of *Klebsiella pneumonia* that was resistant to twenty-six different antibiotics. These antibiotics included representatives from every clinically relevant class, in effect meaning that the infection could not be treated by antibiotic therapy. To better understand how chromosomal mutations can lead to antibiotic resistance we have designed a soft agar gradient evolution plate (SAGE plate) for the rapid evolution of antibiotic resistance in susceptible bacteria. In this presentation we will explore our experience with this system, which a focus on resistance to each of the major classes of antibiotic. Where possible resistance will be linked to mutations in key proto-resistance genes, as well as the effect of resistance on bacterial fitness.

AEM 017

Characterization of Novel Type VI Secretion System Effectors in a Plant Commensal Bacterium

Nathan BULLEN, McMaster University, J.C. WHITNEY [1], [1]McMaster University, London ON

*Pseudomonas protegens*is a plant commensal bacterium that is abundantly found in the rhizosphere and is known for its diffusible antibacterial molecules; however, less well understood is the role that contact-dependent interbacterial antagonism pathways play in this process. Contact-dependent antibacterial killing pathways, such as the bacterial type VI secretion system (T6SS), have the potential to be particularly efficacious in this environment given the propensity of this bacterium to grow on contact-promoting surfaces such as the roots and seeds of plants. Previous work showed that *P. protegens* exhibits potent contact-dependent antibacterial activity against other soil-dwelling bacteria and that this activity depends on its T6SS. The T6SS is a macromolecular assembly that spans the cell envelope of gram-negative bacteria and functions to deliver antibacterial toxins into competing microbes. To further understand the molecular basis for T6-dependent bacterial killing by the *P. protegens* T6SS, we surveyed its genome and identified two candidate toxins based on the presence of T6-associated sequence motifs. While chromosomal deletion of

either of these toxins individually had no observable effect on T6-dependent killing, a strain harboring deletions in both genes showed a marked defect in competitive fitness in co-culture experiments.Using *E. coli* viability assays, we show that the proteins encoded by these genes are toxic to bacteria and that toxin activity is found within the C-termini of these proteins. Co-expression of the genes encoded immediately adjacent to each toxin within the *P. protegens* genome rescues *E. coli* growth, indicating that these genes encode immunity determinants that prevent self-killing. Using enzymatic assays on purified protein, we have determined that one of the toxins, Nbe1, possesses potent NADase activity and thus likely causes cell death in competitor bacteria through widespread disruption of catabolic and anabolic pathways in the cell. In total, our findings begin to describe how the *P. protegens* T6SS is able to kill competitor bacteria and suggest that this pathway may be a significant contributing factor to preventing colonization of plants by pathogenic microorganisms.

II 069

The Mammalian Skin Microbiome

Ashley ROSS, University of Waterloo, J.D. NEUFELD[1], [1]University of Waterloo, Waterloo ON

Skin constitutes the primary physical barrier between mammals and their external environment. Characterization of the microorganisms on skin is essential for understanding how a host evolves in association with its microbial symbionts, modeling immune system development, diagnosing illnesses, and exploring the origins and etiology of disease. Although many studies have characterized the human microbiome, far less is known about the skin microbiome of non-human mammals. The objective of this research was to create a baseline dataset on the skin microbiome of the Mammalia class to determine the effects of species, location, hygiene, body region, and biological sex. The back, torso, and inner thigh regions of 187 non-human mammals and 20 human participants were collected to include representatives from 38 species and 10 mammalian orders. Animals were collected from a variety of locations in Southern Ontario such as local farms, zoos, households, and the wild. All samples were amplified using the V3-V4 16S rRNA gene region and sequenced using a MiSeq (Illumina). The diversity of bacteria and archaea were analyzed from 589 mammalian skin samples, which yielded a total of 22,728 unique operational taxonomic units (OTUs) that were associated with 44 prokaryotic phyla. An indicator species analysis determined that human samples have elevated levels of *Staphylococcus epidermidis*, *Corynebacterium*, and *Propionibacterium acnes* (Table 2), compared to animals that have more soil-related organisms, such as *Arthrobacter* and *Sphinogmonas*. Human skin was significantly less diverse than all other mammalian orders according to Shannon indices (6.54 versus 3.96, $p< 0.001$). The factor most strongly associated with community variation for all samples was whether the host was a human (PERMANOVA, $F = 37.8$, $p < 0.001$; Figure 2). By analyzing all samples together, random forest modelling identified that human and animal samples could be distinguished correctly 98.5±1.2% of the time. The effects of mammalian taxonomy, body region, and location were analysed to elucidate the role these factors have in influencing the skin microbiome. Overall, the sampled mammalian order had the strongest association with the observed variation within animal skin communities (PERMANOVA; $F = 11.3$,$p < 0.001$). Sampled location, such as zoo or pet owner, had a larger effect than the specific cage or house that the animal inhabited. This study represents the largest mammalian skin microbiome project to date and is the first study to elucidate the skin microbiota for 32 distinct species. Additionally, these findings are the first to demonstrate that human skin is distinct, not only from other primates, but from all 10 mammalian orders sampled. Baseline data on the mammalian skin microbiome has important implications for veterinarian research, conservation strategies, and our understanding of mammalian evolutionary history.

AEM 020

Drivers behind the selection of core Fish gut Microbiome

Subba Rao CHAGANTI, University of Windsor, DD HEATH[1], X HE[1], M ZIAB[1], D SANGHERA[1],
[1]University of Windsor, Windsor ON

The gut microbiome of the host (humans, terrestrial animals, and fish) harbors a great number and variety of bacteria which play an important role in host health via the mediation of a variety of biological processes. However a very little is known regarding how the host and environment interact to shape fish gut communities. In the current study, we shed new light on drivers impacting the core gut microbiome formation of the Fish. We tested 1) impact of the environmental factors including the environmental microbiome 2) interspecies competition 3) impact of host genetics, can drive the ecological and evolutionary dynamics of their host through effects on individual, population, community and ecosystem levels. Ion Torrent sequencing was used to characterize the gut microbes by targeting the 16S rRNA gene amplicons. Results will be discussed how each selected factor has its influence on the individual level to the ecosystem level.

SYMPOSIUM X: Synthetic (Micro) Biology
Thursday, June 22nd, 2017
10:30 AM - 12:00 PM
(MGCM)

MGCM 003

Metabolite Valves: Dynamic Control of Metabolic Flux for Pathway Engineering

Kristala PRATHER, Massachusetts Institute of Technology (USA)

Microbial strains have been successfully engineered to produce a wide variety of chemical compounds, several of which have been commercialized. As new products are targeted for biological synthesis, yield is frequently considered a primary driver towards determining feasibility. Theoretical yields can be calculated, establishing an upper limit on the potential conversion of starting substrates to target compounds. Such yields typically ignore loss of substrate to byproducts, with the assumption that competing reactions can be eliminated, usually by deleting the genes encoding the corresponding enzymes. However, when an enzyme encodes an essential gene, especially one involved in primary metabolism, deletion is not a viable option. Reducing gene expression in a static fashion is possible, but this solution ignores the metabolic demand needed for synthesis of the enzymes required for the desired pathway. We have developed “metabolite valves” to address this challenge. The valves are designed to allow high flux through the essential enzyme during an initial period where growth is favored. Following an external perturbation, enzyme activity is then reduced, enabling a higher precursor pool to be diverted towards the pathway of interest. We have designed valves with control at both the transcriptional and post-translational levels. In both cases, key enzymes in glucose metabolism are regulated, and two different compounds are targeted for heterologous production. We have measured increased concentrations of intracellular metabolites once the valve is closed, and have demonstrated that these increased pools lead to increased product yields. We have also incorporated quorum-sensing circuits into the valve design, enabling fully autonomous triggering of flux regulation. These metabolite valves should prove broadly useful for dynamic control of metabolic flux, resulting in improvements in product yields.

MGCM 056

Mutation-Based Conformational Analysis of a New S-Adenosylmethionine Riboswitch Structure in *Burkholderia Thailandensis*

Vesta KORNIAKOVA, INRS-Institut Armand Frappier, B. SELLAMUTHU[1], X. YANG[1], F. KHALFAOUI[1], R.M. NAGHDI[1], J. PERREAULT[1], [1]INRS-Institut Armand Frappier

Riboswitches are structured RNA elements typically located in the 5′ UTRs of bacterial mRNAs and are characterized by their ability to switch ON or OFF a nearby gene by directly binding a specific metabolite and causing a conformational rearrangement responsible for their activity. Riboswitches are composed of an aptamer ligand-binding domain and a variable mechanism-mediated expression platform controlling gene expression. While riboswitches are believed to adopt two distinct conformations, bound and unbound, thus far only the bound conformations of many riboswitch aptamer domains have been confirmed by crystallography. Following our discovery by comparative genomics and bioinformatics of a new S-adenosylmethionine (SAM) II-clan riboswitch structure upstream of *metK* in many bacteria, we attempted to characterize the unbound conformation of its aptamer domain, as well as the effect of a new additional stem. Based on the hypothesis that the two aptamer conformations are of a competing nature, a series of 20 aptamer constructs, containing various mutations aimed at weakening or strengthening the two structures (unbound and bound) were tested for their affinity for SAM by *in-line* probing. Our results indicate that not only do the strengthened unbound structures have a reduced binding affinity for SAM, as predicted, but that the weakened unbound structures also exhibit this reduction. Binding affinity decreases for all mutants by a value ranging between 100 to over 1000 fold compared to the wild-type K_d of 0.3 µM. Furthermore, the alteration or deletion of the new additional stem modestly decreases SAM binding affinity 10 fold. Our results indicate that the unbound structure does not compete with the bound structure contrary to our hypothesis; rather it appears to be a key intermediate in SAM binding. Our study provides insight into riboswitch aptamer mechanics which may help in the engineering of new riboswitches for synthetic biology applications.

AEM 136

The development of a comprehensive CRISPR-Cas9 toolkit for *Bacillus subtilis,* and its application to strain engineering for biomanufacturing purposes

Adam WESTBROOK, University of Waterloo, CP CHOU[1], M MOO-YOUNG[1], [1]University of Waterloo, Waterloo ON

Application of the CRISPR-Cas9 system has dramatically altered the course of genomic engineering across the spectrum of life. *Bacillus subtilis* is a sought after industrial organism and the development of a CRISPR-Cas9 toolkit for scalable strain construction is essential for its progression towards full industrial utility. This work entails the development of a CRISPR-Cas9 toolkit for comprehensive genetic engineering in *B. subtilis*, and extends to the application of the toolkit to advanced metabolic engineering of *B. subtilis* for enhanced hyaluronic acid (HA) production. In addition to site-specific mutation and gene insertion, the toolkit enables continuous genome editing and multiplexing, and is extended to CRISPRi for transcriptional modulation. The toolkit employs chromosomal expression of Cas9 and chromosomal transcription of gRNAs using counter-selectable delivery vectors to facilitate gRNA eviction. Significant improvements to HA titer were obtained by altering expression of enzymes involved in central metabolism via CRISPRi. Moreover, the expected decline in molecular weight of HA produced in recombinant strains of *B. subtilis* was delayed when redirecting carbon flux away from the pentose phosphate pathway. Our toolkit significantly expands the capacity for engineering of *B. subtilis*, and the results of the case study demonstrate the utility of CRISPR-Cas9 technology for optimizing carbon flux through competing metabolic pathways to enhance target metabolite production without compromising host viability.

AEM 031

Semi-Rational Evolution of the RhlA Enzyme from *Pseudomonas aeruginosa* for the Synthesis of Industrially Relevant rhamnolipids.

Carlos Eduardo DULCEY, INRS-Institut Armand Frappier, Y. LOPEZ DE LOS SANTOS[1], E. DÉZIEL[1], N. DOUCET[1], [1]INRS-Institut Armand Frappier

Rhamnolipids (RLs) are glycolipidic compounds produced by a few of bacterial species, especially *Pseudomonas* and *Burkholderia* spp. These compounds display excellent surfactant properties and environmental advantages. Nevertheless, their high production cost hampers their practical use in industry. RhlA acts as a key enzyme in the RL biosynthesis pathway. This enzyme catalyzes the esterification reaction between two units of 3-hydroxylated fatty acids to form a dimer, β-3-(3-hydroxyalkanoyl) alkanoic acid (HAA), the dilipid precursor of RLs. HAA biosynthesis is the rate-limiting step in the carbon flux toward RL biosynthesis; therefore, our research consists in engineering RhlA from *Pseudomonas aeruginosa*UCBPP-PA14 (PA14) for a more effective HAA production, which should result in increased RL production. In order to identify residues that increase the catalytic efficacy of PA14 RhlA, we took a three-step semi-rational evolution approach. First, (1) we opted to predict residues that could interact with the substrate as ligand binding site residues. These residues were first subjected to alanine-scanning mutagenesis. As expected, when any of these single mutants were expressed in a polar PA14Δ*rhlA*strain, the *in vivo* RL production decreased and in some cases disappeared. Given the loss of activity, a secondary round of mutagenesis was used to evolve the enzyme toward increasing activity. These residues were mutated by residues having similar physicochemical properties. The single mutant M37L showed 25% increase in RL production when compared to the wild-type enzyme. Then, (2) we chose potentially relevant residues based on an RhlA homology model. We selected polar residues that seem to be topologically located in close proximity to the predicted RhlA cap-type domain. Selected residues were subjected to site-directed mutagenesis in which they were replaced by amino acids with similar physiochemical properties. We identified eight mutants that increased RL production *in vivo* in minimal salt medium supplemented with glycerol as sole carbon source after 72 h of culture. (3) The next step in our research consists in combining the various substitutions identified in previous steps. Here we report a semi-rational protein evolution approach that enables an increased RL production *in vivo* in *P. aeruginosa*. Nine engineered RhlA variants were able to increase carbon flux toward HAA biosynthesis and, consequently, a higher RL production. As of yet, we have generated RhlA variants which reached 1.5-fold improvement in RLs compared with the wild-type RhlA. This study provides evidence that protein engineering approaches may be successfully used for RL production improvement and similar studies could be extended to modify the RL congener profile and for optimizing proteins involved in the RL metabolic pathway facilitating high-yield RL production in bacteria.

SYMPOSIUM XI: Viruses, Vaccination & Health
Thursday, June 22nd, 2017
10:30 AM -12:00 PM
(I&I)

II 002

Phage morons modulate pathogenesis of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic pathogen that is particularly problematic in Cystic Fibrosis (CF) lung infections, with 70-80% of patients chronically colonized by their teen years. Clinical isolates from CF patients usually contain one or more phages, and many of the bacterial phenotypes involved in CF lung adaptation have been correlated with the presence of prophages. Phages have been shown to affect toxin production, biofilm formation, twitching and swarming motility, mucoidy, and serotype conversion. In the highly transmissible Liverpool Epidemic Strain, proteins expressed from three of its five prophages were shown to strongly enhance its *in vivo* competitiveness in a chronic rat lung infection model. It is clear that phages play important roles in pathogenesis in CF patients; they confer phenotypes that make the infections variable and difficult to treat, and as a result of their mobility they modulate the virulence of *P. aeruginosa* populations in a manner that is currently very difficult to predict. The phage genes that lead to these changes in bacterial pathogenesis are highly variable and are not required for the life cycle of the phage itself. They are collectively referred to as accessory genes or "morons" as their presence adds "more on" the phage genome. We currently lack knowledge of the function of the vast majority of these morons. In this work we examined the influence of phage morons on phenotypic traits that are known to be important mediators of chronic infection in CF patients, and identified a number that influence virulence factor production, biofilm formation, and type IV pilus function. These studies illuminate new mechanisms by which phages mediate bacterial pathogenesis in *P. aeruginosa* and ultimately will help address the important general question of how the ecology of phages in the human microbiome influences disease outcomes.

AEM 088

Validation of UV Inactivation of Enteric Viruses in drinking water and wastewater

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Ultraviolet light irradiation (UV) is commonly employed for the inactivation of enteric viruses in the treatment of municipal drinking water and wastewater. Assessments to determine the performance or efficiency of these systems is often conducted using surrogates for human enteric viruses, such as MS2 bacteriophage. The correlation between the inactivation of MS2 bacteriophage and that of human enteric pathogens, such as adenovirus and rotavirus, are poorly understood; particularly at high doses of UV (>90 mJ/cm^2). The objectives of this study were to: 1) Determine the inactivation of MS2 bacteriophage, human adenovirus strain 41 and human rotavirus by low pressure (LP) UV disinfection units with water at UV transmittance (UVT) values between 50 to 100%. 2) Compare culture- and molecular based assays for the quantification of infectious human viruses in water before and after UV treatment. 3) Evaluate the recovery of viruses from nanoceram filters for the purposes of concentrating viruses in treated water. A small pilot-plant was built to validate the performance of wall-mounted UV disinfection units. Municipal groundwater was seeded with MS2 bacteriophage and infectious adenovirus and rotavirus. UVT was adjusted by adding various amounts of instant coffee to achieve the target values. The UV units were tested with one and two

units in series to elucidate whether virus inactivation was additive during plug-flow. Treated water samples were assayed directly and following concentration by nanoceram filters; hence, the recovery of all viruses from the nanoceram filters was also assessed. All samples were analyzed using culture- and molecular-based techniques. Culture based techniques for the detection of human enteric viruses can be labour intensive, however they provide a measurement of the infectious fraction present in a water sample. Molecular approaches included conventional qPCR, as well as the use of PMA-qPCR (with propidium monoazide) and LA-qPCR (long-amplicon) to determine if these qPCR modification could provide a better estimate of the infectious viruses remaining after treatment, and provide an indication of the level of risk to human health. The preliminary results suggest that: 1) the order of virus inactivation by UV from most sensitive to least was: rotavirus > MS2 bacteriophage > adenovirus; 2) two UV units in series did not provide additive inactivation of the viruses assayed; and 3) the use of PMA-qPCR and LA-qPCR can reduce the qPCR signal from inactivated viruses. The data produced from this study will be valuable for the design of low pressure UV treatment systems for meeting virus water quality criteria and the protection of public health

II 036

In vitro attenuation of a virulent swine isolate of "_Brachyspira hampsonii_"

Janet HILL, University of Saskatchewan, JBDS PEREZ[1], C FERNANDO[1], R NOSACH[1], JCS HARDING[1], [1]University of Saskatchewan, SK

The re-emergence of mucohaemorrhagic colitis in Canadian pigs has been largely attributed to "*Brachyspira hampsonii*", a newly recognized member of the *Brachyspira* genus. Mechanisms of "*B. hampsonii*" pathogenesis are not well understood, limiting opportunities to develop control measures, including vaccines. Our objective was to attenuate a virulent strain of "*B. hampsonii*" to create a potential live attenuated vaccine candidate, and to identify genomic determinants of virulence. A virulent clinical isolate of "*B. hampsonii*" (D09-30446 passage 13, "P13") was passaged in the laboratory 100 times to produce strain P113, which was characterized by biochemical phenotyping and whole genome sequencing. Virulence of P113 relative to P13 was assessed in a mouse model. In each of two experiments, 6-week old CF1 mice were orally inoculated with P13 (n=8), P113 (n=8) or sterile media (n=4). In the second experiment, a fourth group (n=8) was inoculated with a mouse passaged isolate (MP1) from the first experiment. Fecal consistency was the primary outcome, assessed twice per day using a scoring system of 0 (normal feces) to 4 (mucoid feces with blood). No differences were identified in morphology or in diagnostic biochemical markers between P13 and P113 but higher growth rates indicated that P113 was more adapted to laboratory culture conditions than P13. No major chromosomal changes were identified by random amplified polymorphic DNA profiling. Mapping of P113 genome sequence reads on to the P13 genome resulted in identification of 8 SNPs (nonsynonymous substitutions with at least 20x coverage and 70% frequency) in genes encoding proteins involved in transcription, nutrient transport, and a hypothetical gene of unknown function.In the first inoculation experiment, mice in the P113 group had significantly fewer fecal score observations >1 compared to the P13 group, and this difference bordered on significance in the second experiment. There was no difference in fecal score observations of mice in the P113 and MP1 groups in the second experiment. Taken together, our results demonstrate that *in vitro* passage of a virulent "*B. hampsonii*" isolate resulted in reduced virulence in a mouse model of infection. Further passage of the partially attenuated strain through a mouse did not result in additional change in virulence. Serial passage resulted in lab adaptation, with no major genome rearrangements or deletions. Elucidation of the biological significance of any of the SNPs identified in the whole genome sequence comparison will require characterization of the genes affected. Results of this work will lead to identification of virulence factors that may offer targets for further attenuation of "*B. hampsonii*" and the eventual development of vaccines for control of this production-limiting pathogen.

II 072

Discovery and Development of New Host-Targeted Antiviral Drugs

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Influenza A virus (IAV) requires host protein synthesis machinery to create viral proteins. The stress of viral infection can cause protein synthesis arrest and formation of cytoplasmic stress granules (SGs) that entrap viral RNAs. IAV defeats this antiviral response through the action of three viral proteins, but there is a window of opportunity early in infection when pharmacologic induction of SGs can block viral translation. Host-targeted SG-inducing drugs may present a high genetic barrier to resistance. This will be determined by serial passaging of IAV in MDCK cells in the presence of SG-inducing drug, which permits evaluation of potential drug resistance mechanisms. Resistance is a major problem with our current arsenal of antivirals, highlighting the need for improved antiviral therapy. A novel SG-inducing drug (SG-3) is being evaluated in a small animal model of infection. The BALB/c mouse infection model is well established; intranasal infection with mouse-adapted H1N1 IAV, and daily administration of SG-3 altered the immune response in this model, compared to saline control. The immune response and the corrosponding pathogenesis of the virus was analyzed using multiplex, histology, and flow cytometry with and without SG induction. This model allows us to analyze SG induction *in vivo* and the effect on the immune response against IAV and allows future optimization of dose and mode of delivery for candidate drugs.

II 046

An Evolutionary Arms Race: Identification of the first HIV-1 Antagonist against the Interferon Induced Antiviral Protein, HERC5

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From macro, phenotypical change to minuscule, genotypic variation, diversity is what drives evolution. In the human immunodeficiency virus (HIV-1), one of the most variable proteins is envelope (Env). In addition to being essential for binding and entering cells, Env counteracts innate antiviral proteins. Moreover, prolonged passage of HIV-1 in immune cells produces Env mutants that overcome antiviral restriction. A high rate of mutation during replication produces functional, diverse viruses able to target the host immune system. The emergence of these viral mutants within a patient allows for rapid evolution and adaptation to dynamic host conditions. Conversely, the intrinsic response to viral infection is the release of interferon stimulated gene, HERC5. HERC5 has been evolving under strong positive selection for >413 million years, placing it at the forefront of the virus-host interface in vertebrates. We showed that HERC5 inhibits HIV-1 particle production by two mechanisms: HERC5 inhibits nuclear export of incompletely-spliced HIV-1 RNA through the Rev-dependent pathway and blocks an early step in the assembly of the virion at the plasma membrane. Despite potent HIV-1 restriction by HERC5 *in vitro*, infected individuals fail to control HIV-1 replication even though HERC5 is highly expressed during acute and chronic infection. This begs the question: does HIV-1 evolve an antagonist of HERC5 *in vivo*? To determine if divergent Env proteins antagonize the ability of HERC5 to block Rev-dependent nuclear export and/or viral assembly, we independently co-expressed two different strains of Env with HERC5 and demonstrated that one variant of HIV-1 Env antagonized HERC5 and fully rescued viral particle production, while the other had antagonistic ability. We also showed HERC5 and Env interact. We took the study one step further and examined if biologically-relevant Env sequences antagonize HERC5. To investigate this, we developed a yeast-based cloning strategy to efficiently clone patient-derived Env sequences into aa HIV construct. Env sequences from HIV-1-infected individuals in Uganda and Zimbabwe were individually cloned into the HIV-1 plasmid.

Co-expression of these constructs with or without HERC5 resulted in differences up to 4-fold in particle production. Interestingly, 2 of 6 patients screened thus far exhibited full rescue of particle production, 3 of 6 exhibited rescue of nuclear export (mechanism 1) but not particle release (mechanism 2), and 1 of 6 exhibited no rescue in particle production. Our findings have identified the first potential antagonist of the restriction factor HERC5. Furthermore, we have demonstrated the importance of sequence conservation in the observed antagonism. All together, our data will be utilized to identify a novel target for the design of small molecule inhibitors to thwart the viral antagonism of HERC5.

SYMPOSIUM XII: Microbes in Unusual Environments
Thursday, June 22nd, 2017
10:30 AM - 12:00 PM
(AEM)

IS AEM 003

Something from nothing? The creation of a new microbial ecosystem 2500 meters below the surface

Kelly WRIGHTON, The Ohio State University, R DALY[1], M BORTON[1], B O'BANION[1], S WELCH[1], D COLE[1], M WILKINS[1], [1]The Ohio State University, Ohio USA

The terrestrial deep biosphere is one of the least explored and understood ecosystems on Earth. Here, we leverage shale cores prior to and produced fluids after natural gas extraction activities to understand how hydraulic fracturing, the technology for effective gas and oil recovery in deep shales, alters microbial processes or ecology in the deep subsurface. Despite prior reports from more shallow shale systems, our combined microbial biomarker analyses failed to identify signatures for active microbial life in shales prior to energy extraction. Instead, we show hydraulic fracturing injects surface microorganisms 2,500 meters deep into the subsurface, exposing organisms to high pressures and brine-level salinities. Here we use an integrated meta-omic platform to identify microbial adaptations to this new environment. Using laboratory studies, we confirm microorganisms adapt to increase salinity via the production of osmoprotectants, and that excretion of these metabolites fuels an interconnected methylamine network yielding biogenic methane. Microorganisms participating in this food web (osmoprotectant synthesis, fermentation, and methanogenesis) are core members in Appalachian shale wells, suggesting this metabolic network is critical to life in deep shales. Extending beyond microorganisms, we also show that viruses play a critical top down and bottom up controller in this newly synthesized shale ecosystem. Extensive CRISPR links between viruses and hosts, as well as new spacer incorporation in host genomes over time, suggest active ongoing viral predation. In the laboratory, we demonstrated that prophage become induced under known environmental stressors in the fractured shale environment, lysing host cells and potentially releasing osmoprotectant metabolites that sustain microbial metabolism. Collectively this research highlights the resilience of microbial life to adapt to and colonize a new habitat structured by abiotic and biotic stressors far different than their origin.

AEM 016

Soil factors influence archaeal ammonia oxidizers but not methanotrophs in arctic polar desert soils.

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Polar deserts are a vast, 1 358 000 km2, barren, (less than 5% plant cover), xeric, Arctic ecosystem with CH4 and N2O emissions similar to mesic Arctic ecosystems dominated by heaths or willows. It is not clear how the microbial communities of these polar deserts are linked to these unusual soil conditions or to the production of greenhouse gases. Here, we investigated the links between methane-oxidizing bacteria and ammonia-oxidizing archaea, soil environmental conditions, and patterns of net gas production using community-composition DNA microarrays and structural equation modelling across three Arctic polar deserts, located between 77° and 82° N latitude. Surprisingly, ammonia-oxidizing bacteria were not found in sufficient abundance to support detailed analysis, while their archaeal counterparts were found throughout the study area. Methane-oxidizing bacteria were significant drivers of observed patterns of CH4 production, but did not vary with edaphic factors such as organic carbon or total nitrogen. In contrast, ammonia-oxidizing archaea did not drive patterns of N2O production, but were responsive to edaphic factors. Despite this edaphic dependence, neither methane-oxidizing bacteria nor ammonia-oxidizing archaea differed between sites. In this study, N2O production was not linked to archaeal or bacterial nitrifiers, though bacterial denitrifier abundance was too low to analyze. These results highlight two key uncertainties in the biogeochemistry of Arctic climate change modelling: (1) drivers of methanotrophic activity and prevalence ability to promote plant growth and suppress pathogens. The plant protective activity of this bacterium is due in part to its ability to secrete in xeric Arctic soils are not known, and (2) the biological source of N2O in deserts is unknown.

AEM 022

The Impact of Water Quality on the Root-Associated Microbial Communities of Wetland Plants

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Constructed wetlands have become increasingly important as a means of tertiary wastewater treatment in concurrence with processing through wastewater treatment plants. However, optimization of this process has been limited by our lack of understanding surrounding the relationships among wetland organisms involved in the removal of contaminants from wastewater. Two types of organisms that are vital to the process of contaminant removal from wastewater in wetlands are plants and soil microorganisms, specifically bacteria associated with the roots of wetland plants. In order to better understand the relationships between wetland plants and root-associated bacteria, I tested the hypothesis that the microbial rhizosphere communities within wetland systems highly impacted by anthropogenic activities subjected to poor water quality inputs would differ from those communities in areas less impacted by anthropogenic activity receiving higher water quality inputs. To test this hypothesis two riparian sites were selected along the Grand River, one experiencing high anthropogenic impacts and one experiencing low anthropogenic impacts, low water quality and high water quality respectively. Samples were obtained from the rhizosphere and rhizoplane of *Iris versicolor*, *Veronica spicata* and *Potemageton natans* along with water samples from the river at each of the sites over two sampling dates in October 2015. Samples were subjected to culture based analysis using R2A media (total heterotrophic plate counts) and mFC media (total fecal coliforms). The structures of the microbial communities were determined using a combination of denaturing gradient gel electrophoresis (DGGE) and Real-Time PCR. The functional profiles of bacterial communities were determined by

inoculating Biolog EcoPlates™ to assess the ability of each microbial community to use 31 different carbon sources. Structural and physiological differences in the wetland-associated microbial communities present at each site were observed, however the magnitude of these differences was plant species dependent.

AEM 024

Microbial Carbon Transformations in the Western Arctic Ocean revealed through Analysis of Metagenome-Assembled Genomes

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The Arctic Ocean (AO) receives ~10% of the world's river water, resulting in the greatest load of terrestrial dissolved organic matter (tDOM) of any ocean on a per volume basis. This tDOM may serve as a significant nutrient and energy source for the AO bacterial community. However, tDOM is relatively recalcitrant to bacterial degradation and requires specialized enzymatic machinery not typically associated with marine bacteria. In this study we hypothesized that AO communities are enriched in taxa and metabolic pathways capable of tDOM utilization compared to bacterial communities in other regions of the global ocean. To test this hypothesis, comparative metagenomics was performed on bacteria collected from the low salinity surface layer and layers corresponding to the subsurface chlorophyll maximum and the humic-rich coloured dissolved organic matter (cDOM) maximum in the Canada Basin of the AO. Several hundred metagenome-assembled genomes (MAGs) were binned based on tetranucleotide frequencies and differential coverage. MAGs exhibited a strong vertical partitioning across water layers. Many MAGs exhibited a specific association with the cDOM maximum, including those from the Chloroflexi. The Chloroflexi MAGs were assigned to the marine SAR202 clade as well as a novel clade not commonly found in marine ecosystems. All Chloroflexi MAGs possessed genes involved in degradation of humic-like organic matter. Two of these MAGs possessed multiple aromatic compound demethylases. These demethylases were phylogenetically diverse, suggesting these bacteria can target a wide array of methylated aromatic compounds. All but one of the Chloroflexi MAGs possessed numerous flavin-dependent monooxygenases that may be involved in breakdown of recalcitrant organic compounds. All Chloroflexi MAGs also contained genes encoding a variety of carbohydrate active enzymes, including genes involved in the hydrolysis of cellulose, xyloglucans, and xylans. In total, these results support the hypothesis that TOM serves as a source of nutrients and energy for marine Chloroflexi and that the bacterial transformation of humic-like organic matter may be an important contributor to carbon cycling in the AO.

AEM 131

Dynamics of Under-Ice Microbial Community in Freshwater Lakes: Reconstructing Metabolism to Gain Insight About Ecological Roles

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Freshwater lakes in northern temperate and boreal climatic zones play an important role in the global carbon cycle through the storage of organic carbon in their sediments and the emission of carbon dioxide to the atmosphere. These northern lakes are seasonally ice-covered for a large part of the year and it was traditionally thought that microbial communities and processes lay dormant under the ice due to cold temperature and low light level. However, there is now a growing interest in the ice-covered period of northern lakes and in questions about how the metabolic traits and activities of winter communities contribute to lake carbon and nutrient cycling. In this project, we investigated temporal dynamics of microbial community structure and metabolism in a set of temperate and boreal lakes in Eastern Canada,

using metagenomic and metatranscriptomic approaches. Over 350 metagenome-assembled genomes (MAGs) ranging in completeness from 25-92% were binned based on tetranucleotide frequency and differential coverage. Genome diversity under the ice was distinct compared to the open water period. Remarkably, about 15% of the MAGs were members of the Verrucomicrobia phylum, many of which were well represented in the under-ice microbiome. Metabolic reconstruction of Verrucomicrobia MAGs revealed a core genome consisting of metabolic pathways involved in amino acid biosynthesis, degradation and utilization of carbon compounds. Further investigation of carbon-degrading potential of Verrucomicrobia showed significant differences between the number of glycoside hydrolases (GHs) between Verrucomicrobia phylogenetic subdivisions. Interestingly, GHs involved in chitin, cellulose and fucoidan degradation were associated with distinct environmental conditions, suggesting higher degradation rates of specific compounds at different times of the year. Overall, we resolved Verrucomicrobia's phylogenetic diversity at a higher resolution than previously studied and gained insight about their ecological roles in northern freshwater lakes via metabolic reconstruction. Overall, this project provides a first portrait of seasonal dynamics microbial communities in temperate and boreal lakes, and serves as an important baseline study for understanding how future environmental change will affect community structure and their associated ecosystem functions.

Section SYMPOSIUM:
Applied & Environmental Microbiology
Friday, June 23rd, 2017
10:30 AM - 12:00 PM

AEM 027

Microbial Biogeography of the Grand River reveals Strong Spatial Gradients and Flow-Dependent Allochthonous Microorganisms

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The Grand River watershed is the largest catchment in Southern Ontario. The upstream northern portion is influenced primarily by agriculture, whereas the central region receives wastewater effluent and urban runoff. Using same-day samples collected from along the full 300-km length of the Grand River within two different flow seasons, this study investigated in-river bacterial communities as they relate to distance and flow rates. High-throughput sequencing and multivariate statistics of amplified 16S rRNA genes helped us assess the influence of water column physicochemical parameters on the river microbiome. The results demonstrated a consistent shift in detected microbial communities along the river continuum, with dissimilarity increasing with distance from headwaters, hydrodynamic features of the river (i.e., lake and dam), and discharge. Although temperature and anthropogenic input into the river correlated with microbial community variation in both the low- and high-flow seasons, the beta diversity of all spring high flow samples was much less than the beta diversity of the same sample locations at a low flow fall sample time point. This seasonal variability appears linked to higher soil inputs into the river during the spring high flow sampling date. This study provides a microbial baseline for the Grand River, which helps us understand factors influencing microbial assemblages within impacted freshwater systems.

AEM 040

Evidence for Rapid Adaptation during Clonal Expansion of the White Nose Syndrome Pathogen, *Pseudogymnoascus destructans*, in North America

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Research question: White Nose Syndrome (WNS) is an ongoing epidemic affecting multiple species of North American bats, caused by epidermal infections of the psychrophilic filamentous fungus, *Pseudogymnoascus destructans* (optimal growth at 14°C). Since its introduction from Europe in 2006, WNS has spread rapidly across eastern North America and resulted in bat population collapses with mortality rates as high as 95-99%. At present, the mechanisms behind its spread and adaptation to different geographic and ecological niches remain unknown. The objective of this study is to examine the potential evidence for adaptation among strains from selected environments. **Materials and Methods:** We obtained strains of *P. destructans* from multiple locations across eastern North America. Selected strains were tested for their phenotypes in colony morphology, growth rate, spore production, pigmentation, and the viability of spores to survive at high (23°C) and low (4°C) temperatures. In addition, two phenotypically divergent strains from that of the original clone were sequenced. The phenotypic differences were then analyzed together with the genomic differences to identify potential novel mutations that might be associated with their phenotypic

differences. **Results:** We found significant phenotypic variations among clonal descendants of *P. destructans* in North America. Specifically, we found evidence for: (1) phenotypic changes over a geographical gradient, (2) adaptation to spore survival at sub-optimal temperatures, and (3) candidate mutations for adaptive phenotypic changes within the clonal descendants of the fungus. **Conclusion:** Our results show that even though this deadly fungus arrived North America only recently, there has been significant microevolution and adaptation during its short expansion in the new geographic niche

AEM 048

Time-Series Clustering of Marker Genes Reveals Ecological Dynamics

Michael HALL, Dalhousie University, R.G. BEIKO[1], [1]Dalhousie University, Halifax NS

Marker-gene sequencing has quickly become an invaluable tool for investigating microbial communities. The resulting sequences provide insight into the composition and diversity of a community, allow us to infer its functional capabilities, and assess the dynamics as the community responds to its environment over time. A typical approach makes the data more tractable by collapsing similar sequences, often using a 97% sequence identity cut-off. The resulting clusters of sequences are termed “operational taxonomic units” (OTUs). We suggest a complementary approach: collapse sequences that demonstrate similar distributions across samples. To that end, we developed Ananke, a software package for clustering and visualizing longitudinal marker-gene data sets. In place of OTUs, our algorithm produces sets of “time-series clusters” (TSCs), which are groups of sequences that exhibit similar dynamics in their relative abundances over time. Given the independence of TSCs from sequence similarity, distantly related organisms can be grouped together if they share common temporal dynamics, signalling potential interactions or shared ecological niches. For example, TSCs of a temperate lake data set reveal that seasonal cyanobacterial blooms are coincident with blooms of the *Bacteroidetes* lineage bacI, both of which are known occur under conditions of high dissolved organic carbon. Conversely, our method can reveal closely related sequences (such as those that belong to the same OTU) that display distinct temporal dynamics, suggesting that the commonly used 97% sequence identity threshold can be too coarse. Ananke distills large sets of marker gene sequences into sets of distinct temporal profiles. These sets can be used as a foundation for data exploration, time series modeling, and forecasting.

AEM 108

The Ecology of Methylotrophic Bacteria in Aquatic Environments

Arthi RAMACHANDRAN, Concordia University, DA WALSH[1], [1]Concordia University, Montréal QC

Methylotrophy is the ability of bacteria to use one-carbon (C1) compounds as a source of carbon and/or energy. Methylotrophic bacteria are widespread in nature and are found in a variety of aquatic habitats. They play an important role in the cycling of carbon in marine and freshwater ecosystems. In this study, we investigated the diversity, distribution, and metabolism of a group of beta-proteobacterial methylotrophic bacteria (OM43/LD28 clade) in a variety of freshwater and marine environments in North America. The OM43/LD28 clade is comprised of three distinct subclades, each exhibiting specific environmental distribution with OM43 dominating marine environments and LD28 dominating freshwater ecosystems. We discovered that the OM43/LD38 clade of methylotrophs are widespread and abundant and show temporal and spatial variation linked to seasonal patterns and salinity. Metagenomic analysis of the Western Arctic Ocean revealed a distinct Arctic subclade specific to the surface waters providing evidence for specific adaptation and ecological specialization of the OM43/LD28 clade. 16S rRNA analysis showed higher relative abundance in the winter compared to the summer in three limnologically distinct Quebec lakes. Querying metagenomic and metatranscriptomic data using a functional gene marker provided evidence that these bacteria are not only present in these lakes but are also active. Ongoing metagenomic and metatranscriptomic analyses of these different ecosystems will increase our knowledge with regards to the

other metabolic traits and activities associated with methylotrophy. Comparative metagenomics of marine and freshwater methylotrophs from the Arctic Ocean and the Quebec lakes along with other available metagenomics datasets will provide insight into the evolutionary relationship between these two populations and begin to help us understand the complex nature of habitat transition.

AEM 111

Probiotic Fermented Food for 120,000 People in Africa, and Counting.

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In 2004, we initiated a project in Mwanza, Tanzania with the intent of providing the means for local women to produce yogurt supplemented with probiotic *Lactobacillus rhamnosus*GR-1. The reasons were several-fold. The HIV/AIDS crisis was taking its toll on the health and economic viability pf families, in particular due to morbidity and mortality of males. Many infected, even those on anti-retroviral therapy, suffered from chronic diarrhea and fatigue, conditions which could potentially be alleviated by probiotics. Despite rapid growth in global sales of probiotics, companies were absent from Africa due to poor distribution networks and the inability of people to afford the products. Our concept was to teach local women's groups how to make probiotic yogurt in their community. The highly documented GR-1 strain was donated for this purpose. Studies had shown this strain could inhibit the growth of intestinal and urogenital pathogens, and when delivered in yogurt it could modulate immunity, in some cases elevating the CD4 count in HIV patients, and reduce diarrhea and fatigue. Thirteen years later, this program has expanded to reach over 120,000 consumers daily. The scientific advances that made this possible were the identification of a *Streptococcus thermophilus*C106 strain that could ferment milk and produce an excellent tasting product; plus, the ability of probiotic strains *L. rhamnosus*GR-1 or *L. rhamnosus* Yoba (a generic version of LGG the world's most documented probiotic) to replace *L. delbreuckii*subsp. *bulgaricus*as the other starter culture; and reach suitable viable counts that confer a variety of benefits on the host. In addition, a sachet with a particularly effective seal was created to retain viability of the two strain combinations (C106 + GR-1, or C106 + Yoba) for two years. A label was created to provide instructions on how to make the fermented food. Each sachet costs the producers US$0.50 and allows them to make up to 100 litres of the food. An IDRC grant has allowed the project to extend to Uganda, Kenya and southern Tanzania, where an extensive and growing network of community kitchens (production units) now distributes the food. Profits are retained by the producers to cover marketing, expansion and family income, often allowing children to attend private schools. We have recently shown that the sachets can also be effective at producing fermented millet. This is important as the product is nutrient-rich, and utilizes a grain widely available in the region due to its drought and heat resistance. During dry season or in parts of the world where milk is scarce, the use of millet can extend the outreach. Overall, the project illustrates the power of microbial metabolism and utilization of local resources to confer health and economic benefits to poor populations. The ability to translate our science to the real world need not be as far away as we think.

AEM 140

Case study on the impact of antibiotic treatment on gut microbiota of preterm infants with and without NEC

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Neonatal necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease that affects preterm infants. Once diagnosed, treatment options are aggressive, usually involving intensive antibiotic therapy or surgical intervention. Though NEC pathogenesis is not yet well-defined, drastic microbiome changes preceding clinical onset suggest that the early gut microbiome has a direct role in NEC onset. We hypothesize that the preterm gut microbiome is essential for healthy GI development, and when the functional integrity of the ecosystem is disturbed (including thorough use of antibiotics), this leaves the infant vulnerable to NEC. In this study, we aim to differentiate NEC and non-NEC microbiomes based on compositional and functional characterizations. To do this, preterm fecal microbiota were cultured in a gut environment-mimicking continuous bioreactor. Subsequently, a combination of microbiological culture methods, and 16S rRNA gene and metabolomic analyses were applied to comprehensively isolate, identify and characterize the composition and behaviour of fecal ecosystems derived from NEC and non-NEC infants. The resulting *in vitro* ecosystems were subjected to an antibiotic treatment that recapitulated the typical drug dosages administered to preterm patients. The compositional and metabolic responses to this perturbation were used to evaluate the functional robustness of each ecosystem. The functional disparities between NEC and non-NEC microbiomes highlighted by this study provide reference points for understanding the behaviour of the preterm microbiome in NEC and non-NEC cases, and its response to typical antibiotic treatment.

II 006

Enzyme immobilization on biomaterial surfaces for prevention of *pseudomonas aeruginosa* biofilms

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Introduction: Biomaterial surfaces are at significant risk of developing bacterial biofilmassociated infections. Approximately 60% of all hospital acquired infections (HAIs) are the result of biofilm formation on biomaterial surfaces and the bacterium *Pseudomonas aeruginosa* is one of the most prevalent isolated pathogens. Bacterial biofilms are communities of bacteria encased within a protective extracellular matrix. Exopolysaccharides (EPS) are the predominant component of biofilm matrix for many bacteria, contributing to initial adhesion, architecture, and resistance. Bacteria in biofilm are highly tolerant to antibiotics and can evade the host immune system. Recently we identified and produced several recombinant glycoside hydrolases that selectively target and hydrolyze biofilm EPS. Two of these enzymes $PslG_h$, and $PelA_h$ hydrolyze the Psl and Pel polysaccharides from *P. aeruginosa*, respectively, and were found to be effective in solution at disrupting the biofilms from lab, clinical and environmental isolates. Current antibiotic therapy and antimicrobial surfaces are ineffective against biofilm infections. Therefore, we aim to use surface - immobilized enzymes as a means of preventing biofilm formation on biomaterials. **Materials and Methods:** We tested the immobilization of $PslG_h$ onto polymer biomaterial surfaces as a means of preventing biofilm growth for *P. aeruginosa* in long term static and flow culture. The enzyme was covalently bound with glutaraldehyde crosslinking to glass and PDMS surfaces and its attachment confirmed by ATR-FTIR and contact angle measurements. To evaluate antibiofilm efficacy, our biomaterial surfaces were incubated in *P. aeruginosa* culture that overexpresses the Psl polysaccharide for varying duration. Fluorescence microscopy and fluorescent dyes (SYTOX Green) were used and for quantitative analysis, we used turbidity and crystal violet staining methods. **Results and Discussion:** The covalentlybound $PslG_h$ inhibited both cell attachment and biofilm formation of the *P. aeruginosa* (Psldependent) cells, compared with the control (i.e., no enzyme) or a catalytically inactive enzyme (over a 24 hour period. This indicates that the antibiofilm activity of PslGh is directly related its catalytic activity. The covalentlyimmobilized $PslG_h$ enzyme was also effective at preventing biofilm formation for an 8 day static culture. Quantitative analysis using crystal violet straining indicated significant antibiofilm activity for the immobilized PslG, as compared with the control sample. **Conclusion:** These results indicate the high specificity of PslGh in targeting the Psl exopolysacchride in *P. aeruginosa* biofilms, which appears to greatly inhibit bacterial colonization and biofilm growth, even when the enzyme is covalentlyimmobilized on the polymer surface. *D. Asker is also affiliated with Food science and Technology Department, Alexandria University, Egypt

II 031

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

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Intracellular bacterial pathogens are capable of causing a diverse array of diseases in humans and represent a significant threat to global health. These pathogens have evolved sophisticated strategies including the release or secretion of virulence factors to interfere with host cell functions and to perturb immune responses.

In this context, quantitative proteomics has recently matured into a powerful technology to comprehensively characterize mammalian cells, as well as bacteria, on the level of proteins. However, their interplay in the context of infection has not been systematically investigated. Our 'infectome' analysis aims at the identification of previously undescribed proteins involved in bacterial virulence as well as host immune defense, representing an opportunity to elucidate molecular mechanisms of host-pathogen interplay during disease. Here, we investigate the host-pathogen interplay between the pathogenic bacteria, *Salmonella* Typhimurium, and bone-marrow-derived macrophages. We performed quantitative proteomics of the host cells infected with wild-type (SL1344) or the type 3 secretion system (T3SS) mutant (dspi-1 and dspi-2) strains in single runs using high resolution mass spectrometry on a QExactive HF Quadrupole Orbitrap instrument. Overall, we provide a comprehensive and dynamic view of both pathogen and host proteins during infection. In the host cells, we observed the upregulation of proinflammatory proteins (i.e. interleukin and interferons) and lysosomal proteins, representing host defense mechanisms to initiate immune responses and combat bacterial invasion. For *S.* Typhimurium, integration of proteome and infectome data identified proteins not encoded on the T3SS pathogenicity islands as being co-regulated with known virulence factors, suggesting a co-functional role in virulence and infection. In addition, we observed an increase in expression of *pdu* operon proteins in the dspi-2 strain suggesting a novel connection between pathogenicity and metabolism. Furthermore, comparison of our proteome profiles to available transcriptomic datasets revealed correlation between proteins and their transcripts, in addition to suggesting roles in virulence. Current work is pursuing the mechanistic characterization of Spi1- and Spi2-associated candidate pathogenic proteins. Overall, our approach represents an innovative strategy to comprehensively characterize and elucidate molecular mechanisms of host-pathogen interplay during disease from both host- and pathogen-centric perspectives.

II 043

Denosumab, a Monoclonal Antibody to Receptor Activator of Nuclear Factor-Kappa B Ligand, Modulates the Gut Microbiome Dysbiosis in the Context of Experimental Colitis.

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Background: The pro-inflammatory mediator receptor activator of nuclear factor-kappa B ligand (RANKL) plays a major role in the development of rheumatoid arthritis; however, its role in inflammatory bowel disease is unknown. Genome-wide association meta-analysis for Crohn's disease (CD) identified a variant, near the TNFSF11 gene that encodes RANKL. Moreover, CD risk allele increased expression of RANKL in specific cell lines. This study aims to elucidate how the RANKL inhibitor (Denosumab) by modifying gut microbiota dysregulation can be harnessed to diminish experimental colitis and ultimately serve as a safe therapeutic target in IBD. **Methods:** CD-like colitis was induced via intrarectal administration of dinitrobenzene sulfonic acid (DNBS, 4mg/kg) dissolved whether in ethanol (30%) or PBS (1%) to C57Bl/6 mice (n=24/group). One day before colitis induction, daily injection of denosumab (10mg/kg/day, i.p.) was initiated and continued over four days. Control mice received either ethanol (30%) or PBS (1%) in both groups of with or without denosumab treatment. On the sacrifice day, inflammatory status was evaluated clinically. DNA was extracted from colonic tissue and fecal samples, and V4 region of bacterial 16S rRNA gene was amplified and subjected to Illumina sequencing for microbiome analysis. Alpha- and beta diversities were calculated in QIIME and subjected to SAS and PERMANOVA, respectively. Differences in clustering at the genus level were determined using Bray-Curtis dissimilarity clustering analysis. Differences ($P < 0.05$) between treatments were tested using two-way ANOVA followed by the Tukey's multiple comparisons in Prism. **Results:** Disease severity, macroscopic score and pro-inflammatory cytokines (Il-6, IL-1β, and TNF-α) were increased in DNBS/ethanol-treated vs. control mice. Denosumab treatment decreased only the pro-inflammatory cytokines ($P < 0.05$). Denosumab treatment also modified the alpha-

and beta- diversities of colonic and fecal microbiota. While DNBS/ethanol-treated animals clustered separately compared to the control group ($P < 0.05$), denosumab treatment attenuated the negative effects of DNBS/ethanol resulting in clustering of these samples closer to the controls. The predicted functional capacity of bacterial community was significantly altered following denosumab injection in DNBS/ethanol-treated group including but not limited to increase in cell growth signaling P53 pathway as well as genetic replication and repair pathways. **Conclusions:** The development of colitis in DNBS-ethanol model was accompanied by disruption of gut microbiota. Preventative treatment with denosumab modulated intestinal inflammation and gut microbiota dysbiosis in a murine model of colitis. This provides a rationale for considering denosumab as a therapy in CD.

II 051

Investigating the ability of *Lactobacillus sp.* to reduce *Candida albicans*-induced immunopathology

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Fungi infect billions of individuals, resulting in the death of over 1.5 million people per year. Capable of causing both superficial and life-threatening disease, *Candida albicans* is the most common human fungal pathogen. With the understanding that clinical use of antibiotics can increase the risk of subsequent fungal infection, several studies have identified the ability of *Lactobacillus sp.* to provide prophylactic treatment against *C. albicans.* The purpose of this study was to investigate the interactions between host, *C. albicans* and *Lactobacillus sp.* in order to understand microbial dynamics and how they affect the outcomes of infection. We determined that *C. albicans* filamentation in response to host-relevant cues was blocked during a co-culture with *Lactobacillus rhamnosus*. Additionally, we achieved this inhibitory effect in a dose-dependent manner when we treated *C. albicans* with *L. rhamnosus* conditioned media. Through a high-throughput imaging screen of the *C. albicans* Gene Replacement and Conditional Expression (GRACE) library, we identified two genes *RGT1* and *HAT2,* which were required for this block in filamentation in response to *L. rhamnosus* conditioned media. Finally, we determined that *L. rhamnosus* is able to reduce the rate of pyroptosis of murine macrophages in response to *C. albicans* infection. Overall, we have identified new complexities in the dynamic relationship between mucosal communities and host-immune responses.

II 080

Enterohemorrhagic *Escherichia coli* responds to gut microbiota metabolites by altering metabolism and activating stress responses

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The mammalian gastrointestinal tract is a fiercely competitive environment for the bacteria that inhabit it. Pathogenic bacteria attempting to colonize the gut must compete with the established community of microbes known as the gut microbiota. The gut microbiota inhibits pathogen colonization by numerous mechanisms, including competition for limited nutrients and production of metabolites and cell-associated structures that directly inhibit pathogen growth. Nevertheless, pathogenic bacteria such as enterohemorrhagic *Escherichia coli* (EHEC), which causes severe bloody diarrhea, are able not only to colonize the gut but also to proliferate to high numbers. We hypothesized that EHEC has evolved specific responses that enable it to survive and reproduce in the presence of competing gut microbes. Here, we used a combined RNA-Seq and Tn-Seq approach to identify EHEC genes that are involved in the adaptation to growth in the presence of gut microbiota-produced metabolites. For these experiments, EHEC was grown in the presence of cell-free supernatant from an *in vitro*-grown, defined community of 33 human gut bacterial isolates. Using RNA-Seq,

we identified 117 EHEC genes that were upregulated and 44 that were downregulated in the presence of gut microbiota metabolites, while Tn-Seq analysis identified 23 EHEC genes that increase fitness and 5 genes that decrease fitness under these conditions. Overall, our data revealed that EHEC adapts to growth in the presence of gut microbiota metabolites in two major ways: by altering its metabolism and by activating stress responses. Metabolic pathways that improved fitness in the presence of microbiota metabolites included systems for maintaining redox balance (such as UdhA, which is responsible for reoxidizing NADPH) and the NtrBC nitrogen limitation response. Conversely, EHEC reduced its expression of biotin biosynthesis genes when exposed to microbiota metabolites, reflecting the large amount of biotin secreted by the microbiota into their culture medium. In addition, numerous stress response genes were either upregulated (envelope stress response genes *cpxP* and *spy*, oxidative stress response gene *soxS*, and poorly characterized stress response genes *yhcN* and *bhsA*) or important for fitness (including the EF-P lysyltransferase genes *epmA* and *epmB*) during EHEC growth in the presence of microbiota metabolites. Taken together, these results suggest that EHEC optimizes its survival and growth in the presence of competing gut microbes by adjusting its metabolism to make use of microbiota-produced metabolites such as biotin, while simultaneously activating stress responses to defend itself against damaging molecules produced by the gut microbiota. An improved understanding of pathogen-microbiota interactions may ultimately enable the development of therapies to enhance colonization resistance in order to prevent infection by enteric pathogens.

II 084

Attenuated *Salmonella typhimurium* as a vector for a novel *Clostridium difficile* vaccine

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Attenuated *Salmonella enterica* species are attractive as vaccine vectors due to their potential to induce both local (mucosal) and systemic immune responses. To facilitate stimulation of immune responses, type III secretion systems (T3SS) of *Salmonella* can be employed to deliver heterologous antigens to antigen-presenting cells. The genome of *S. enterica* contains two loci termed Salmonella pathogenicity island 1 and 2 (SPI-I and SPI-II) that encode distinct T3SS that translocate effector proteins at the different stages of Salmonella infection. While these secretion systems have been exploited previously to deliver foreign antigens in *Salmonella*-based vaccine development efforts, the distinct spatial and temporal functions of the SPI-I and SPI-II systems on immune responses, particularly in terms of mucosal immunity, have yet to be systemically investigated. Our initial antigenic targets are the C-terminal receptor binding domains (RBDs) of *Clostridium difficile* toxins A and B (TcdA, TcdB). Anti-RBD antibodies have been shown to protect against *C. difficile* infection in both animal models and humans. To date, we have developed a panel of 13 vaccine candidates based on a well-characterized, attenuated *S. typhimurium* strain (YS1646) that express the RBDs of either TcdA or TcdB using different SPI-I and SPI-II promoters and secretory signals. Western Blot and immunofluorescence results show that expression of these antigens is variable *in vitro*, both when the bacteria is grown in LB broth and upon invasion of a mouse macrophage cell line (RAW264.7). Preliminary data in a mouse vaccination model (3 doses of 10^9 bacteria by gavage either every other day or every 2 weeks) suggest that several of these vaccine candidate that exploit different SPI-I and SPI-II T3SS promoters and secretory signals elicit systemic immune responses at least (IgG by ELISA). We are currently optimizing the vaccine schedule to find the constructs that elicit both systemic and mucosal immunity (serum IgG, stool fluid IgA, cellular responses). Once optimized, vaccinated animals will be challenged with a lethal dose of *C. difficile* to assess the efficacy of protection. These studies will shed light on the use of SPI-I and SPI-II T3SS to deliver heterologous antigen as well as the potential of *S. typhimurium* strain YS1646 as a vector – the known safety profile of this bacterium will likely increase the chance of bringing a novel *C. difficile* vaccine to clinical trials.

Section SYMPOSIUM:
Molecular Genetics & Cellular Microbiology
Friday, June 23rd, 2017
10:30 AM - 12:00 PM

MGCM 005

Cell Envelope Protein Interaction Map in Escherichia Coli

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Knowledge of cell envelope protein (CEP) complexes is vital for a mechanistic understanding of bacterial membrane assembly, antibiotic resistance and metabolic coordination, yet only limited characterization of relevant macromolecules has been reported to date. I will present a systematic global proteomic survey of CEPs encompassing most of the inner- and outer-membrane and periplasmic proteins of Escherichia coli K-12. After extraction with mild non-denaturing detergents, we affinity-purified 816 endogenously-tagged CEPs and identified stably-associated polypeptides by precision mass spectrometry. The resulting high-quality physical interaction network, comprising over half (58%) of all targeted CEPs, revealed hundreds of previously unknown heteromeric complexes. These include novel transporters and other broadly-conserved multi-component assemblies mediating membrane protein export/folding, outer membrane biogenesis, multidrug resistance, and the mechanistic links coordinating nutrient uptake with metabolism. The broad biological and evolutionary significance of the CEP 'interactome' provides mechanistic insights into the molecular landscape of essential bacterial CE systems, facilitating antimicrobial development.

MGCM 013

Conflict can Enhance Bacterial Community Survival: The Case of Cross-Feeding and Colicins in a Defined Community of *E. Coli* and *Salmonella*

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Colicins are toxic proteins that kill cells lacking an antitoxin, but their ecological functions and impacts on community interactions are understudied. To study the ecological role of colicins, we developed a two-species community wherein *E. coli* is the only species able to access a lactose carbon source, making *Salmonella* dependent on *E. coli* to excrete nutrient byproducts. *Salmonella* produces colicins that kill *E. coli*, creating a paradox in which *Salmonella* absolutely requires *E. coli* to produce food but simultaneously kills *E. coli* with a toxin. In an attempt to crash the essential *E. coli* population, an iron chelator was added to the community to enhance colicin production. Surprisingly, the community grew better at higher chelator concentrations than *E. coli* did alone. Polymicrobial RNA-seq revealed that *E. coli* upregulates genes for the maintenance of electron transport functions in the cell envelope, suggesting that colicins trigger a stress response that inadvertently helps *E. coli* survive severe iron limitation. Further, titrating colicins into *E. coli* cultures allowed us to isolate a genetically resistant subpopulation of *E. coli*, which DNA sequencing revealed to contain a mutation in iron and colicin uptake. These results reveal that competition with a colicin can enhance community survival in a simple cross-feeding experiment between *Salmonella* and *E. coli*.

MGCM 021

Activation of a Cryptic Glycopeptide Antibiotic-Related Biosynthetic Gene Cluster

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Sequencing of microbial genomes reveals them to be a rich source of biosynthetic gene clusters encoding the production of bioactive specialized metabolites. As many of these clusters are transcriptionally silent under standard laboratory culturing, understanding their regulation is critical. Here, we describe the characterization of a cryptic cluster, *gp6738,* from an environmental *Streptomyces* isolate. The *gp6738* cluster resembles those of clinically relevant glycopeptide antibiotics (GPAs) such as vancomycin, encoding a non-ribosomal peptide crosslinked by cytochrome p450s and rich in nonproteinogenic aromatic amino acids. RT-PCR revealed that it is transcriptionally silent in the endogenous host as well as in a heterologous host, *Streptomyces coelicolor* M1152. An StrR-like regulator, GP6738-10, was identified at the border of the cluster, and three putative inverted-repeat binding sites were located inside the cluster. Through the overexpression of GP6738-10 along with another putative regulator, GP6738-9, the cluster was activated in *S. coelicolor*. RNAseq analysis of this strain was used to characterize the role of these transcription factors in regulating the cluster, as well as predict the cluster's borders and operonic structure. The co-activation of the entire cluster despite the lack of predicted GP6738-10 binding sites in front of several operons highlights the role of crosstalk with other regulators. Understanding this cluster's regulation will aid in the isolation of its product, as well as in the activation of related clusters. Indeed, all known GPA clusters contain an StrR-like regulator, and we have identified >5 other cryptic GPA clusters containing a GP6738-10 homolog. Activation of these cryptic clusters offers the potential to mine their chemical matter for clinically relevant bioactivity.

MGCM 029

Cell wall stress sensing enables resistance to killing by cell wall acting antibiotics in *Vibrio cholerae*

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Most bacteria are surrounded by a cell wall, which is crucial for maintenance of cell shape and integrity. The main component of the cell wall is peptidoglycan (PG), a polysaccharide web crosslinked via peptide bridges. This rigid mesh has to simultaneously sustain tremendous intracellular pressures while also allowing for modifications such as controlled expansion (during growth) and the insertion of large macromolecular protein complexes. Cell wall synthesis and modification have to be tightly balanced; this is evidenced by the well-known consequences of inhibition of cell wall biogenesis (e.g. by exposure to beta lactam antibiotics), i.e. cell wall degradation and typically cell death. How this tight balance is maintained is largely unknown. Here, we show that Vibrio cholerae, the Gram-negative causative agent of cholera disease, does not die after inhibition of cell wall synthesis. Instead, if forms viable spheres that readily revert to rod-shape after withdrawal of the antibiotic. A genetic screen for requirements for sphere to rod reversion was answered by the major PG synthase Penicillin Binding Protein 1A (PBP1A). Consistent with a major role in sphere survival, PBP1A levels were increased ~10fold in response to inhibition of cell wall synthesis by beta lactam antibiotics. PBP1A upregulation depended on the presence of a novel histidine kinase/response regulator system we have named WigK/R (for wall integrity gauge). RNAseq experiments revealed that besides PBP1A, the WigK/R regulon comprised the entire cell wall synthesis pathway. We suggest that WigK/R is a cell wall damage sensor that responds to imbalances between cell wall synthesis and degradation by increasing the capacity for cell wall biogenesis.

MGCM 086

A CRISPR Cas9-Based Gene Drive Platform for Studying Complex Genetic Interactions in *Candida albicans*

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Fungal pathogens have emerged as a leading cause of human mortality, particularly among the growing population of immunocompromised individuals. The frequency of invasive fungal infections has increased by over 200% in recent years, with staggering economic and public health costs. The opportunistic pathogen *Candida albicans* reigns as the leading cause of fungal infection and the fourth most common cause of hospital-acquired infection, with mortality rates approaching 50%. However, genetic analysis in this clinically important fungal pathogen remains cumbersome due to its unusual codon usage, inability to retain plasmids, and diploid genome. Here, we have developed a novel CRISPR-Cas9-based genome-editing strategy for rapid, precise, and highly efficient genome editing in *C. albicans*, with exciting applications for global genetic analysis of fungal pathogenesis. In our system, the RNA-guided Cas9 endonuclease is used to delete *Candida* genes with very high targeting efficiency. Further, by changing the architecture of the donor DNA molecule being delivered to replace the native genomic locus targeted by Cas9, we have created a 'gene drive': a selfish genetic element which will readily replace the targeted site,and,upon mating,will further propagate, and replace the incoming wild-type locus at high-efficiency. As such, we have engineered a system to easily create homozygous deletion mutants in a single experimental step. Coupling this approach with newly identified mating-competent haploid lineages of *C. albicans*, we are able to quickly and efficiently create diploid *Candida* strains that are double homozygous deletion mutants - deleted for any two genes of interest. This technology marks a significant advancement over previous genetic techniques in *C. albicans*, and facilitates our ability to create double knock-out mutants in an unprecedentedly high-throughput manner, to perform synthetic genetic analysis, and assess genetic interactions on a large scale. We demonstrate the power of this technology by generating two double-gene deletion libraries, each comprised of 55 or 78 unique *C. albicans* strains, deleted for two genes of interest, involved in drug efflux, or cellular adhesion, respectively. Screening these libraries has revealed new insight into combination factors involved in key fungal virulence processes, including antifungal drug resistance and biofilm formation. Together this work revolutionizes our ability to uncover genetic circuitry regulating *Candida* pathogenesis, ultimately helping to identify factors that may serve as therapeutic targets for treating fungal infections.

MGCM 098

A Broadly Distributed Toxin Family Mediates Contact-Dependent Antagonism Between Gram-positive Bacteria

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The Firmicutes are a phylum of bacteria that dominate numerous polymicrobial habitats, including the human skin and gut microbiomes. Interbacterial competition is fierce within these densely inhabited environments; however, broadly distributed contact-dependent antagonistic mechanisms utilized by Gram-positive Firmicutes have not been elucidated. Here we show that proteins belonging to the widespread LXG polymorphic toxin family mediate cell contact- and Esx secretion pathway-dependent antagonistic interactions between Firmicutes. The Streptococci are a genus of Firmicutes composed of commensals and pathogens of mammals. We identified LXG proteins secreted by *Streptococcus intermedius* that potently inhibit the growth of bacteria within and outside of the genus. The structure of one such toxin revealed a previously unobserved protein fold that we show directs the degradation of a uniquely bacterial molecule

required for cell wall biosynthesis, lipid II. Although the Esx secretion systems of several bacteria have been functionally characterized, a generalizable role for the pathway has remained elusive. Our phenotypic and biochemical data linking LXG toxins to Esx broadly implicate this pathway in antagonistic interbacterial interactions. Its wide target specificity and prevalence across the Firmicute phylum suggest Esx plays a key role in shaping microbial community structure, including in the many Firmicute-rich communities associated with the human body.