Poster Competition Abstract

Tuesday, June 19th, from 3:00 - 5:00 PM

Presenting students are to be by their posters from **3:00 – 5:00 PM** to respond to judges' questions.

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

AEM PC 01

Isolation and Characterization of Novel Microorganisms from the Canadian High Arctic using the Cryo-iPlate

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The Earth's biosphere is predominantly cold, with as much as 80% permanently below 5°C. Among these cryo-environments are the Polar Regions, which includes the Canadian high Arctic. Despite the inhospitable conditions these regions present, cold-adapted microorganism (cryophiles) have been shown to be living at the low temperature limits of life. These microbes can inform us on many basic research questions concerning the fundaments of life. While molecular techniques can provide certain information on cryophiles, the pure sequence data these techniques provide is insufficient to inform us on new gene or pathway function. A true understanding of the adaptations, physiology and metabolic capacities of microorganisms requires their cultivation in laboratory settings. However, it is well known that over 99% of bacteria cannot be cultured using standard cultivation techniques. Advanced cultivation techniques attempt to overcome this limitation by culturing microbes *in situ.* One such example is the cryo-iPlate. Based on the ichip, the cryo-iPlate is designed for the high-throughput cultivation of cryophiles in their natural environment. Here, we propose to use the cryo-iPlate to isolate, identify and characterize a collection of highly novel cryophilic microorganisms. Cryo-iPlates were set up in unique polar habitats surrounding the McGill Arctic Research Station (MARS) in the Canadian high Arctic. Following two weeks of incubation, the iPlates were returned to McGill University in source-sample containing whirlpack bags and left to incubate at -10° C for an additional three to four months. Two iPlates incubated in Gypsum Hill hummock active layer soil have been opened and processed: a pure gellan gum plate (GHAL) and a supplemented media plate (SMHAL). Preliminary results have shown the iPlate's ability to culture diverse microbes from extreme cryo-environments. A total of 98 and 124 morphologically distinct microorganism isolated from the GHAL and SMAL plates respectively have been grown in pure culture on standard artificial media in petri dishes. These isolates are currently undergoing 16s rRNA sequencing in order to determine their phylogeny and novelty. The adaptations, physiology and metabolic capacities of selected novel cryophilic strains will subsequently be characterized through metabolic and growth assays (above- and sub-zero temperatures), in addition to whole genome and transcriptomic sequencing (Illumina MiSeq). The iPlate plugs that do not lead to microbial growth on artificial media will be treated as *in situ* enrichment cultures and analyzed through metagenomic sequencing (Illumina MiSeq) followed by genome binning. Based on the successes of the cryo-iPlate prototype and the original ichip method in isolating novel microbial strains, the cryo-iPlate has the potential to greatly increase the number cryophilic microorganisms in pure culture and, thus, enhance our understanding of the cold temperature limits of life on Earth.

AEM PC 02

Exploring the cellulolytic microbial diversity in landfills through metagenomic and cultivation approaches

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Microorganisms' diverse metabolic capabilities allow adaptation to varied environments, which may be leveraged for biotechnological applications. Industry applications seek factors such as increased enzymatic efficiencies and product yield for large-scale processes. Understudied, complex, engineered environments such as municipal waste sites (MWS) host highly novel and diverse microbial communities degrading waste and conducting functions of interest for biotechnology applications. The uncharacterized microbial diversity and functions in landfills may hold novel cellulose-degrading communities and activities useful for improvements in enzymatic degradation of cellulose for biofuel production. This research characterizes the capacity for cellulose degradation in 2 MWS. Metagenomes from 2 MWS separated by geography and climate were generated. Firstly, the leachate and adjacent river metagenomes from a dump in Kingston, Jamaica were analyzed for their microbial composition and the potential for cellulose degradation within the microbial community members. A total of 23 high quality metagenome-assembled genomes (MAGs) were binned, with 7 phyla represented. The majority of MAGs belong to the Bacteroides, Proteobacteria, and Firmicutes, with 1 MAG representing a Candidate Phylum Radiation lineage. In the leachate and river metagenomes, 0.11% and 0.08% of the total coding sequences were affiliated with 5 GH families containing cellulases, encoding domains from 6 Pfam protein families. Secondly, metagenomic and culture-dependent methods were used in tandem to assess cellulolytic potential in a landfill in Southern Ontario. Three metagenomes from leachate wells, 2 from a composite leachate cistern, and 1 from a groundwater well in the adjacent aquifer were analyzed, examining gene abundance of proteins belonging to GH families containing cellulases and the taxonomy of these genes. Overall, genes belonging to GH3 were in greatest abundance across all 6 metagenomes. An overwhelming majority of the potential cellulase genes were annotated as from members of the Bacteroidetes. Biomass collected from filtered cistern leachate was grown in synthetic leachate medium supplemented individually with copy paper, cardboard, newsprint, and filter paper, enriching for cellulose degraders. Sequencing of 16S rRNA genes from a time-course of these enrichment cultures identified cellulolytic microbial populations from the landfill, whose *in situ* abundance was determined from the landfill metagenomes. As complex, heterogeneous, and multiply-contaminated sites, they hold great potential for the discovery of novel genes, microbes, and functions valuable for biotechnology and bioremediation. Our work determined the cellulolytic potential at 2 MWS and developed active enrichment cultures with novel cellulolytic organisms. Further characterization of these landfill-derived cultures and MAGs may identify industrially-relevant enzymes and expand the known cellulolytic organisms.

AEM PC 03

Characterization of the microbiome of the thick-billed murre and its association with prey specialization and mercury

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Seabirds are used to monitor contaminants in the ocean because they cover large areas while feeding and bring the environmental signal back to a central location, their colonies, where they can be easily sampled. Individual prey specialization, occurring in many seabirds (such as the thick-billed murres, *Uria lomvia*), should be taken into consideration when using wildlife as bioindicators, given that diet plays an important role on the concentrations of contaminants that are accumulated by wildlife. Such specialization may influence the composition of the gut microbiome, as bacteria inhabiting the intestines of prey specialists will vary among diets. Studying how the gut microbiome, individual prey specialization, and Hg interact with each other will allow us to expand our knowledge of the ecotoxicology of thick-billed murres and how they integrate different levels of food webs. We present the first documentation of the gut microbiome of an Arctic seabird using 16S rRNA gene profiling. The murre gut microbiome appears to be dominated by the phyla Firmicutes and Proteobacteria. The description of individual prey specialization patterns allowed us to identify three main types of diets, with combinations of them also present for some individuals. We found no significant differences between Hg concentrations among the types of diet. We suggest that quantifying diet composition using next generation sequencing of the 12S rRNA mitochondrial gene could elucidate differences that cannot be observed with the categories we identified.

AEM PC 04

Response of the soil bacterial community to crops and weediness levels from a long term field experiment

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Plant species and community composition in addition to edaphic factors are great driving forces on microbial community structure and function. This is because plants are the primary providers of specific carbon and energy sources through exudates and litter which are readily available to soil microbes for mineralization. Modern agroecosystems are largely characterized by monoculture with weeds species being the source of aboveground plant diversity. There is a lack of information on how crops grown in rotation and with different weed densities influence soil microbial communities. The objectives of this study were to determine how different crops grown in rotation and weediness levels influence soil microbial communities over time. Research was initiated in a long term fully phased field study at the Ian N. Morrison Research Farm located in Carman, Manitoba. The study consists of an annual crop rotation, with three levels of selective in-crop herbicide applications and two controls (fallow and prairie) arranged as a RCBD with three replicates. A total of 27 phyla groups were detected in all the bulk soils by illumina sequencing with *Proteobacteria* and *Acidobacteria* being the most dominant groups. Crop species and weediness levels influenced the bacterial community of the bulk soil over time. Both fallow and prairie clustered differently from each other and also from all other crop treatments on the PCA ordination plots at most sampling dates. Weediness level was important at shaping the bacterial community in most treatments which shifted with sampling date, but its effect was more profound in wheat treatments. The results indicate that aboveground plant species greatly influence the composition of soil microbial communities. Knowledge of relationship between plant diversity and soil microbial communities is essential to understanding the links between aboveground and belowground communities and modulation of ecosystem functioning.

AEM PC 05

Evaluation and characterization of Shiga-toxin- producing *Escherichia coli, Salmonella enterica,* and *Pseudomonas spp.* in multi-species biofilms

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Foodborne illness caused by Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella enterica* represent a major economic burden and concern for public health and safety. Furthermore, these microorganisms form surface-linked aggregates known as biofilms that have been shown to be persistent sources of contamination in food processing facilities. However, previous studies on the ability of these pathogens to form biofilms have been mainly limited to single species, whereas most biofilms formed in nature are composed of multiple species in close interaction. The purpose of this study is to characterize and evaluate the ability of STEC, *S. enterica*, and *Pseudomonas spp.* to form biofilms *in vitro*. Biofilm formation, curli and cellulose production was evaluated for twenty-seven STEC, six *Pseudomonas spp.* and nine *S. enterica* strains. Subsequently, four different combinations of *S. enterica*, STEC, and *Pseudomonas spp.* strains were chosen to be used in multi-species biofilm trials to determine the interaction of the three microbes. Biofilm formation was determined using a Crystal Violet staining assay. Curli and cellulose production was ascertained via media incorporated with Congo Red and Calcofluor dyes respectively. The number of microbes of each species within mono- and multi-culture biofilms was determined by scraping biofilms, serially diluting, and enumerating the number of microbes via plating on media. Each experiment contained two replicates and three independent trials were conducted. A one-way analyses of variance test was used to determine if significant differences existed among the treatment means, and a least significant difference test was used to determine differences between means ($P<0.05$). Strains that produced both cellulose and curli were strong biofilm formers, whereas strains that produced only cellulose or curli had moderate biofilm forming ability, and strains that could not produce cellulose or curli were weak biofilm formers. Three out of the four combinations of *S. enterica*, STEC, and *Pseudomonas spp.* strains showed significant differences between mono- and multi-culture biofilms ($P<0.05$). These significant differences exhibited interactions ranging from commensal, additive to competitive. Enumeration of the microbes within the biofilms further provided validation to the aforementioned interactions, and verified which species had a significant contribution in multi-species biofilms. While most research on biofilms is based on mono-culture biofilms, the fact remains that biofilms in the industry and natural environment are generally composed of multiple species. As such, a paradigm shift from single-species to multi-species biofilm research to accurately represent natural conditions is imperative to resolve substantial gaps in our current understanding of the complex interaction of microorganisms residing in biofilms.

AEM PC 06

Microbiological investigation of lava tube ice for determining its habitability on Mars

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Cave ecosystems harbour very diverse microbial communities. Of these environments, cave cryoenvironments are understudied but could be of great importance to microbiologists as they help determine the microbial limits of life not only in relation to cold temperatures but also combined with constraints such as limited light availability, and low nutrient concentrations. There is also interest in using these environments to study astrobiology and the search for life on Mars. If microbial life exists elsewhere in our solar system it may be more likely to be found in lava tubes on the banks of Martian volcanoes where temperatures are relatively stable, there is protection from solar radiation, water may accumulate as ice and the loss of nutrients from wind and dust storms is minimized. Little is known of the identity or level of activity of the microbes that inhabit ice within cave ecosystems. The goal of my research is to characterize the microorganisms found in lava tube ice to help determine if lava tubes present a suitable habitat for extinct/extant life on Mars. We collected ice samples aseptically from lava tube caves in Lava Beds National Monument, California, U.S.A. (41º42'50" N 121°30'30" W) during the summer of 2017. To get a broad picture of the microbial community in lava tube ice we performed measurements of biomass using plate counts and determined the community composition using MinION metagenome sequencing. The MinION is a miniature nucleic acid sequencer ideal to test with our Mars analog samples because it may one-day launch to Mars, Europa or Enceladus due to its small size and energy requirements. At 5°C, heterotrophic plate counts ranged between $2.23x10^4$ and $2.91x10^7$ CFU/ml in our samples. The temperature preference of the microorganisms in our samples indicate the community of cultured microorganisms is adapted to cold temperatures with most appearing to be psychrophiles. These results match with biomass estimations from other cave cryoenvironments. The microbial composition of our samples is dominated by members of the Bacteroidetes (38%-82%), Proteobacteria (17%-28%), and Firmicutes(1%-23%). Members of these phyla are found in cryoenvironments around the world and may be adapted to cold temperatures, however the major phyla found in our lava tube ice samples differ from other cave cryoenvironments demonstrating cave ice as a novel environment to study diversity. To our knowledge this is the first microbiological investigation of lava tube ice and our results suggest these microorganisms are distinct from other environments and adapted to conditions similar to present day Mars lava tubes.

AEM PC 07

Effect of iron on the characterization and biofilm formation ability of *Salmonella* spp. on spinach

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Salmonella spp. are commonly known human pathogens which are responsible for an increased incidence of food borne illness related to fresh fruits and vegetables. Research has shown that *Salmonella* are able to exist in the environment on crop plants through biofilm formation. The objective of this study was to understand if iron acquisition has an effect on the biofilm formation abilities of *Salmonella* spp. on spinach. Wildtype *Salmonella enteric* Typhimurium and *Salmonella enterica*Enteritidis serotypes and their three complement mutants; a TonB iron transport system knockout, a double siderophore receptor knockout, and a transporter and receptors triple knockout, were used. Several assays were performed to compare the biofilm formation abilities of wildtype and mutant strains. Crystal Violet (CV) biofilm assays were performed at 18ºC for 96h and at 23ºC for 72h and the optical density (OD) of the CV stain was read on a Microplate reader at 595 nm to quantify biofilm formation. To identify curli and cellulose within biofilms, Luria Bertani low sodium (0.5 g/L) plates, containing either 40 μg/ mL of Congo red (CR) and 20 μg/ mL of Coomassie brilliant blue or 20

μg/ mL of calcofluor brightner, were used. Colony morphology was observed on CR plates, and fluorescence under 365nm UV light was observed on calcofluor plates. Scanning electron microscopy (SEM) was performed to visualize the growth of biofilms on spinach leaves after 96h at 18ºC. Biofilms formed on spinach leaves were subjected to a 5% glutaraldehyde fixation, ethanol dehydration and coated in gold for visualization under the microscope. Motility was determined by stab inoculating motility test media containing 0.001% 2, 3, 5 tryphenoltetrazolium chloride, and visualizing after 16h at 37ºC for spread away from the stab. Crystal Violet biofilm assays were repeated 4 times and all other assays were repeated 3 times. Analysis of variance was performed and differences between strains was determined using the Student-Newman-Keuls test, $p=< 0.05$. The CV assay showed the wildtypes to have significantly stronger biofilm formation than TonB mutants at 18ºC. *S.* Typhimurium wildtype and mutant had an OD of 3.01±0.36 and 1.90±0.19, respectively, and *S.* Enteritidis wildtype and mutant had an OD of 0.96±0.18 and 0.58±0.11, respectively. Colony formation on CR plates showed *S.* Entritidis TonB mutants lack curli production but all calcofluor plates were positive for cellulose. All strains showed motility, however, visualization of biofilm formation by SEM showed stronger colonization ability of the wildtype strains. The results of this study have identified iron acquisition as a limiting factor to *Salmonella* spp. growth within biofilms on spinach. This finding has increased knowledge of *Salmonella* spp. biofilm characterization and formation on spinach and has provided a better understanding of how this human pathogen can exist in the environment on plants.

AEM PC 08

Antibiotic Resistant Bacterial Communities in a Municipal Wastewater Treatment Facility

Jennifer RUSSELL, University of Regina, C. K. YOST[1], [1]University of Regina

Antibiotic resistant pathogen-caused infections are one of the biggest medical hurdles of the 21st century, with concerns that we are moving into an era where antibiotics will no longer work against pathogenic bacteria. In order to respond to this threat, scientists are studying environments where antibiotic resistance genes are acquired and transferred, finding that wastewater treatment facilities amplify resistance rates and contribute to the spread of antibiotic resistance genes in the environment. However, the majority of studies are only looking at the resistance gene abundance, and are not establishing the identity of the bacteria housing these genes, which is necessary information when assessing public health risks. This study looked at eight different antibiotic resistant bacterial communities from a newly upgraded Canadian municipal wastewater treatment facility using culturing and 16S rRNA amplicon sequencing. The resulting data demonstrates the plant's excellence in bacterial removal, but highlights the possibility of multi-drug resistant pathogens surviving the treatment process, and being released into the environment.

II PC 01

Characterization of a novel response regulator, NbmR, involved in antibiotic susceptibility and virulence in *Acinetobacter baumannii*

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Acinetobacter baumannii is an opportunistic pathogen posing an increasing risk on global health care settings. Therefore, understanding the molecular mechanisms underlying its pathogenicity is of paramount importance. Here we report for the first time, the involvement of an orphan response regulator (NbmR) in the antibiotic susceptibility and virulence of *A. baumannii*.

II PC 02

Competitive fitness of essential gene knockdowns reveals a broad-spectrum antibacterial inhibitor of the cell division protein FtsZ

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For patients with cystic fibrosis, colonization and infection by bacteria in the *Burkholderia cepacia* complex is strongly associated with mortality. The high levels of intrinsic and acquired antibiotic resistance within the *Burkholderia cepacia*complex necessitate exploration into alternative treatments. To this end, the small synthetic benzothiadiazole C109 was previously identified as a broad-spectrum antimicrobial; however, the target was unknown. To facilitate further investigation, the goal of the current work was to elucidate the target and mechanism of action of C109. Based on the theory that gene knockdown increases susceptibility to cognate antimicrobials, we constructed a library of essential gene knockdown mutants in *B. cenocepacia* K56-2. Using Tn-seq and Illumina sequencing to track individual mutants grown competitively, we validated our approach by showing that mutants with knockdown in DNA gyrase B were more susceptible to novobiocin. When exposed to C109, we detected several susceptible mutants, including those with a knockdown in the *division and cell wall* (*dcw*) cluster. Phenotypic analysis of C109-treated cells showed marked filamentation, suggesting a block in cell division and further implicating the *dcw* cluster. Using GFP-tagged constructs, we observed that C109 prevented assembly of divisome components encoded by the *dcw* cluster, suggesting the essential first-localized protein, FtsZ, may be targeted. Indeed, specific knockdown of FtsZ sensitized cells to C109. To validate the target, *in vitro* analysis revealed that C109 inhibited the critical polymerization and GTPase activities of FtsZ. To explore interactions of C109 with clinically relevant antibiotics, we performed synergy tests and found that C109 acts additively with members of 7 antibiotic classes. Together, these results characterize a novel FtsZ inhibitor with broad-spectrum activity meriting further applied investigation.

II PC 03

Bioluminescent *Salmonella* Strains to Study Host - Pathogen Interaction in Chicken

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Chickens (*Gallus gallus domesticus)* are asymptomatic carriers of *Salmonella enterica* serovar Enteritidis (SE), one of the main *Salmonella* serovars responsible for human salmonellosis globally. Here we have characterized putative virulence genes of SE using a bioluminescent imaging (BLI) model to investigate the role of each gene in colonization & infection in chickens. We hypothesized that BLI would provide insight into host-pathogen interactions between SEand the chicken and further our understanding of *Salmonella* infection, colonization and transmission within the avian host.To allow for continuous monitoring of SE during *in vivo* infection, the lux operon (*luxCDABE*) from *Photorhabdus luminescens* was integrated into the SE wildtype and mutant strains, and expressed under a constitutive promoter to generate a continuous light signal. Virulence genes of interest included: *Salmonella* Pathogenicity Island 1 (SPI-1), SPI-2, ferric uptake regulator (*fur*), *pagN* (i.e., PhoP/Q regulated genes) and *tonB* (which encodes the energy transducer to facilitate Fe^{3+} uptake). Using cell enumeration, we compared virulence-defective mutant strains of SE compared to wildtype cells for differences in their ability to colonize day-old birds following oral challenge. We did not see a significant difference in cecal colonization from either virulence-defective mutant strain compared to wildtype at day 4, 5 post challenge. This was confirmed by *ex-vivo* imaging where strong signals came through cecum from each reporter strain during the time frame. The cecum is thought to be the

primary colonization site for SE in chicken and our finding is contrast to role of SPI-1, *pagN* in mammalian models which play an important role in gastrointestinal colonization. SPI-2 plays a major role in systemic infection in mammals and similarly, we observed a dramatic reduction in the bacterial load in spleen and liver. However, performing enrichment cultures at day 4, 5 post challenge showed that 100% of the birds were systemically infected (most of them at a very low level). Iron is thought to be critical for bacterial survival and TonB facilitates uptake of Fe^{3+} from host tissue. Our data show evidence that Fe^{3+} uptake may not be critical for colonization in the cecum or systemic infection chicken during early life. Fur acts as a global regulator in regulating iron homeostasis in Gram negative bacteria. Disruption of*fur* didn't affect colonization in the cecum yet affected the systemic phase of infection at day 4 post challenge. Overall our approach using BLI has revealed new insights into the interactions between SE and chickens.

II PC 04

Proteomic Delineation of Mosquito and Mammalian Cell Dysregulation after Zika Virus Infection

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Background: The Zika virus (ZIKV) pandemic brought a lot of concerns globally due to lack of information in patient diagnosis and management.Thus, in addition to gaining more basic information about ZIKV biology, appropriate interventions and management strategies are being sought to control ZIKV-associated diseases as well as its spread. **Objective**: To identify host cell proteins that are significantly dysregulated during Zika virus infection and determine their roles in the virus replicative cycle. **Methods**: Quantitative mass spectrometry and aptamer-based SOMAScans were used to identify and measure host proteins dysregulated during ZIKV infections. **Results**: C6/36 mosquito, and mammalian Caco-2, HEK-293, Astrocytoma and Vero cells were infected with ZIKV and cells harvested at various times post-infection. Quantitative mass spectrometry and quantitative aptamer-based SOMAScans identified hundreds of dysregulated proteins that were significantly up- or down-regulated more than 1.25-fold. Western blots confirmed dysregulation of some of these proteins and bioinformatic analyses of these proteins identified their specific cell signaling pathways. siRNA-mediated knock down of some of these genes to determine their effects on ZIKV replication are underway. **Conclusion**: This study identified host proteins that are significantly dysregulated by ZIKV infection and will hopefully identify those that play key roles in the ZIKV replicative cycle. Identification of these proteins provides more basic information about ZIKV biology and they may serve as targets for future interventions.

II PC 05

Discovery of a short proline-rich lipopeptide that enhances the antibacterial activity of minocycline and rifampicin against multidrug- and extensively drug-resistant *Pseudomonas aeruginosa*

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The increasing incidence rate of multidrug- and extensively drug-resistant (MDR/XDR) Gram-negative bacterial infection in hospitals worldwide including *Pseudomonas aeruginosa* impose a serious problem to our healthcare system. Antimicrobial peptides (AMPs) present a viable pool of biologically active compounds for potential therapeutic usage. Proline-rich antimicrobial peptides (PRAMPs) are a class of naturally-occurring AMPs characterized by an unusually high amount of L-proline. Moreover, PRAMPs are amphiphilic with overall cationic character and often possess a repeating motif of PXP or PXXP, where X is any L-amino acid but typically L-arginine. We prepared 16 short proline-rich lipopeptides (SPRLPs) to mimic longer naturally-occurring PRAMPs via solid-phase peptide synthesis and assessed their biological activity against a panel of Gram-positive and Gram-negative pathogens. Lipopeptides under consideration

consist of only seven L-amino acids and a lipid component. Further investigation for the therapeutic usage of these SPRLPs was done on MDR/XDR clinical isolates of *P. aeruginosa*. Out of the 16 prepared lipopeptides, we identified a lead lipopeptide structure that inherently possess limited antibacterial activity alone but significantly potentiate the antibiotics minocycline and rifampicin against MDR/XDR *P. aeruginosa*. Synergism with these two antibiotics is likely due to enhanced membrane permeability induced by the SPRLP. Toxicity assessment of the lead SPRLP revealed that it is non-hemolytic and non-cytotoxic against human liver carcinoma HepG2 and human embryonic kidney HEK-293 cell lines. An initial attempt to optimize the lead candidate through peptidomimetic modification was performed by replacing the L- to protease-stable D-amino acids. The lead candidate structure was found to be pliable to modification and further optimization strategies are currently underway.

II PC 06

Identifying novel anti-infective drugs against *Pseudomonas aeruginosa* using a combined computational and experimental screening approach

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Pseudomonas aeruginosa is a major cause of nosocomial infection in individuals with compromised immune system or cystic fibrosis, and is one of the top three pathogens the World Health Organization lists as urgently requiring new treatment therapeutics. Targeting virulence instead of essential functions potentially generates novel drugs that disarm rather than kill disease-causing bacteria. Such drugs are likely to be more specific to pathogens and subject to less selection for resistance. Using a combined bioinformatics, chemical genomics, and structural systems biology approach, primary analysis predicted Raloxifene, a selective estrogen receptor modulator used for osteoporosis treatment, may also interact with the Pseudomonas PhzB2 protein in phenazine biosynthesis pathway, resulting in a reduced production of the pyocyanin toxin, responsible for inducing oxidative stress in hosts. An infection model showed a dose-dependent improvement of survival in Pseudomonas-infected *C. elegans* treated with Raloxifene. To study the structure-activity relationship of Raloxifene in anti-infective treatment, three side chains of the drug were modified to produce two analogs, Compound 1 (modified hydroxyphenyl and piperidine groups) and Compound 2 (modified piperidine group only). Compound 1 indeed had minimal activity against *P. aeruginosa*infection, while Compound 2 surprisingly had better anti-infective activity than expected: Pyocyanin exotoxin in *P. aeruginosa* was reduced further when treated with Compound 2, compared to Raloxifene. Compound 2 also showed better protection than Raloxifene in the worm infection model. Both Raloxifene and the analogs showed minimal effect on bacterial growth, consistent with proposed anti-infective, instead of antimicrobial, activity. No toxic effects of Compound 2 were noted against *C. elegans*. This study has given insight into the essential structures of Raloxifene, granting its potency as an anti-infective drug against *P. aeruginosa* infections. Repurposing Raloxifene not only showed promising efficacy as an anti-infective, but also our approach enabled the identification of a novel anti-infective compound warranting further study to address the urgent need for new therapies.

II PC 07

Characterization of *Ebolavirus* cell tropism in isolated primary cells and in a 3D blood-testes barrier co-culture model

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Ebola virus disease (EVD) is characterized by widespread organ failure, severe diarrhea and, in some cases, hemorrhagic manifestation. Recently, longitudinal cohort studies have suggested that up to 26% of male EVD survivors during the recent West African EVD epidemic had persistent testicular Ebola virus (EBOV) infections. These persistent infections were not associated with signs of inflammation or illness and it has been suggested that persistence spanned 7-9 months in a quarter of these cases. Further, high concentrations of infective virus have been found in semen, surpassing that found at peak viremia. Alarmingly, there have been several case reports of sexual transmission by persistently infected male EVD survivors. Thus, persistent testicular EBOV infections are a major public health concern for controlling spread and re-emergence of EBOV into the population. The objective of this study is to understand the molecular mechanisms underlying persistent testicular EBOV infections. Here, we employ live and pseudotyped ebolaviruses to investigate in isolated testicular cells and a 3D model of the blood-testis barrier (BTB). We also aim to study testicular cell tropism and persistence across all *Ebolavirus*species. Initial investigations of cell tropism were performed in the murine Sertoli (15P-1) and Leydig (MLTC1) cell lines with pseudotype EBOV-lentiviruses expressing the viral glycoprotein (GP) and Lux or GFP. Subsequent investigations were performed on 3D-transwell cultured 15P-1 cells and primary murine BTB cells. Morphology and tight junctions between murine testicular cells of the BTB model will be examined by fluorescence microscopy immunoblotting and RT-qPCR of specific markers; claudin-11 (tight junction), WT1 (Sertoli), CYP11A1 (Leydig), ED1/2 (macrophages), Chk2 (Germ cells). These markers will be used to confirm primary murine cell identity. Fluorescence microscopy confirmed that both 15P-1 and MLTC1 cell lines were permissive to EBOV entry of pseudotyped EBOV. These results were confirmed with EBOV-GFP infections demonstrating high replication in both 15P-1 and Vero cells, and significantly slower replication in the MLTC1 cells. EBOV cell tropism was confirmed in 15P-1 cells grown under 3D cell culture conditions. Our results suggest EBOV persistence occurs in the testis due to slower replication of EBOV in Leydig cells, allowing only a small amount of EBOV to enter much more replication competent Sertoli cells.

II PC 08

Response of human induced pluripotent stem cells to Influenza infection

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Introduction: As the most common type of fetal malformations, congenital heart defects (CHDs) affect various stages of life, ranging from fetal demise to asymptotic defect in adulthood. Epidemiological data suggest a link between maternal influenza infection and such anomalies in offspring. Nevertheless, the possible mechanisms behind the maternal influenza-induced CHD in the fetus remained unknown. Human induced pluripotent stem cells (hiPSCs) can be considered as a model for studying the effects of influenza virus not only on the early stage of human embryonic development but also on the cardiogenic signaling pathways in the differentiation phase. We hypothesize that influenza virus probably directly disrupts the normal differentiation of embryonic stem cells into cardiomyocytes, potentially leading to CHDs by inhibiting or dysregulating signaling pathways involved in fetal cardiogenesis. **Methods:** After infection with IAV at different MOIs, undifferentiated infected hiPSCs were sorted by FACS, and the cardiac differentiation was induced through the hanging drop method. Histological examination and assessment of electrophysiological parameters were carried out for both infected and control resulting three-dimensionally

(3D) cultured cardiomyocytes. At various post-infection and post-differentiation time points, the IAV replication kinetics and viral mRNA were measured by plaque assay and RT-qPCR, respectively. Western blotting was done to determine the viral protein production. The pluripotency levels of infected hiPSCs were assessed by immunofluorescence microscopy. The SOMAScan analysis was performed to identify IAV-induced changes in the hiPSCs proteome. Differentially expressed proteins were further analyzed by multiple bioinformatic tools for characterizing their potential roles in dysregulating cardiogenic signaling pathways and embryonic development. **Results:** IAV replication was restricted in hiPSCs. However, cell viability decreased significantly at 12 and 24 hpi even at low MOI, and noticeable cytopathic effects were demonstrated in infected cells. Viral proteins were only detectable at MOI of 5. In contrast to limited intrinsic apoptosis, autophagy appeared to become activated by IAV earlier than apoptosis and at much higher levels. Significant alterations were observed in the expression of proteins present in the initial differentiation and cardiac differentiation signaling pathways, such as PDGF, IGF-1, EGF, mTOR. **Conclusion:** IAV affects the normal differentiation of embryonic stem cells into cardiomyocytes via dysregulating the expression of several critical regulators within cardiogenic signaling pathways.

MGCM PC 01

Ribitol catabolism by *Rhizobium leguminosarum* affects competition for nodulation

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The *Rhizobia* are a collective group of soil-dwelling, root and stem-nodulating bacteria to which *R. leguminosarum* bv. *viciae* strain VF39SM is a member of. *R. leguminosarum* bv. *viciae* is agriculturally and environmentally important due to its' nitrogen fixing abilities with agricultural crop plants such as peas, beans and lentils, improving the yields of these crops and at the same time mitigating the use of nitrogen-based fertilizers that pollute nearby water bodies. VF39SM has six plasmids, a-f, which have been found to contain genes for the catabolism of many different carbon sources such as the sugar alcohols erythritol, glycerol and ribitol. Previous research has demonstrated that erythritol and glycerol catabolic gene mutants were less competitive for nodulation of peas compared to the wild-type VF39SM strain, indicating that the uptake and catabolism of these sugar alcohols plays a role in rhizosphere colonization and nodulation competitiveness of VF39SM on pea plants. Southern blot, combined with genomic sequence analysis has detected the presence of two, non-homologous ribitol catabolic gene systems present on pRleVF39 c and d of *R. leguminosarum* VF39SM. The objective of this research is to determine if the ribitol catabolic genes on c and d plasmids of VF39SM play a role in host plant rhizosphere colonization and nodulation competitiveness, as well as the role of ribitol catabolism on the biochemistry of VF39SM. This research will also investigate the operon structures and regulation of both ribitol catabolic gene systems by RT-PCR and *gusA* promoter-fusions in response to ribitol and host plant seed or root exudates. Current results show that the promoter of the regulator on the d-plasmid, *rldR*,and the periplasmic binding protein on the c-plasmid, *rlcA*, are induced by ribitol and host plant seed exudates. A modified Fähraeus slide technique, combined with tissue staining showed that the *rldR* promoter is induced on the rhizoplane of lentils (cv. Indian Head), but that the *rlcA* promoter is not. Nodulation competition assays show that a VF39SM *rlcK-rldD* double mutant that is unable to grow on ribitol, is less competitive than wild-type VF39SM for nodulation of lentils (cv. Marble), but not peas (cv. Little Marvel). The results to date indicate that these two ribitol catabolic gene systems are induced during the host plant-VF39SM interaction, but that the system of genes that is induced is either determined by the host plant species, or by the stage of the VF39SM-host plant interaction.

MGCM PC 02

Functional analysis of a structurally complex ECF anti-σ factor from *Bacillus subtilis*

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Extracytoplasmic function (ECF) σ factor - anti-σ factor systems are one of the three major routes by which bacteria sense and respond to changes in the external environment. These systems respond by regulating transcription, the first step of gene expression, to ensure survival of cells in variable environments. This work investigates the structure and function of RsiO, an integral membrane protein and ECF anti-σ factor of the *B. subtilis* two-subunit σ factor SigO-RsoA. The SigO-RsoA-RsiO regulatory system allows the cell to sense and respond to certain changes in environmental conditions including acidic pH. Transcription activation assays and protein-protein interaction assays demonstrated that RsiO negatively regulates the activity of SigO-RsoA by sequestering SigO from its co-σ factor RsoA and from core RNA polymerase. Characterization of RsiO function using systematic mutagenesis identified two highly conserved charged residues in RsiO likely involved in SigO sequestration. We have also experimentally mapped the predicted topology of RsiO as a N_{in} C_{in} polytopic integral membrane protein with four transmembrane segments, an unusually complex topology for an anti-σ factor. RsiO topology appears to be influenced by growth on acidic medium (pH 5.4) and possibly regulates the activity of SigO-RsoA via the conversion of RsiO topology from N_{in} C_{in} at pH 7 to N_{in} C_{out} at pH 5.4. This scenario could provide the mechanistic basis of SigO release and transcription activation. Anti-σ factor activity is often suppressed by the proteolytic degradation of the protein under inducing conditions. In the case of RsiO, we have not detected proteolysis. This raises the possibility that RsiO may be a direct sensor of hydronium ion concentration and undergoes a topological re-organization as pH decreases. To our knowledge, no other anti-σ factor has been identified as a direct sensor of this physicochemical parameter.

MGCM PC 03

A LacZ reporter for the study of the role of Rgg/SHP homologs in *Streptococcus pneumoniae* Quorum Sensing

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Drug-resistant *Streptococcus pneumoniae* (*S. pneumoniae*) is still listed as a major threat by Centers for Disease Control and Prevention (CDC) despite vaccine usages. Understanding signaling pathways controlling *S. pneumoniae* virulence and commensalism will provide us hints to treat pneumococcal-related diseases. As quorum sensing (QS) is a well-established mechanism by which secreted signaling molecules communicate with neighboring cells to regulate cell behaviors, exploring QS may bring new ideas about disease control. In *S. pneumoniae*, two Two-Component-Signaling-Transduction-System (TCSTS) QS systems have been identified: ComABCDE/CSP controlling competence, and BlpABCHR/BIP controlling bacteriocin production. Rgg/SHP signaling and regulating systems are the third class of QS in *S. pneumoniae.* There are at least eight Rgg paralogs, but their functions are unclear. We hypothesize that each of those Rgg paralogs controls a distinct gene regulon and behavior. To understand these regulation networks, suitable reporter tools are needed. Quantitative RT-PCR directly measures gene expression levels, but is too cumbersome for screening individual colonies at a large scale, a drawback also applies to luciferase reporters. In contrast, the LacZ reporter can be used both for a large-scale colony screening and for quantitative measurements of expression levels in isolated cultures. We, therefore, constructed a LacZ reporter readout tool to explore Rgg/SHP QS, beginning with *rgg0939*. Specifically, a plasmid, pHDY03, carrying a tetracycline resistance marker, a LacZ reporter downstream of the *shp0939*promoter, and two homology blocks flanking the Tet^R-$P_{shp0939}$-*LacZ* cassette, was used to insert the reporter cassette into the neutral locus of the β-galactosidase gene by homologous recombination. Testing the readout of activity for

the *shp0939* promoter by quantitative β-galactosidase assay and X-gal white/blue screen, we found this tool to be reliable and useful. We found that: 1) The endogenous pheromone SHP0939 peptide did not activate the expression of *LacZ*, which implies the existence of an upstream Rgg/SHP regulator. 2) LacZ expression began immediately (>10 minutes) after adding 500nM synthetic SHP3-C8. 3) There was an 80-fold difference of LacZ expression between -SHP and +SHP. 4) The level of expression in the negative control was basal, and the maximal readout was comparable to that of a LacZ driven by a competence inducible promoter. 5) Blue colonies yielded when the CDM agar was supplemented with 100nM SHP3-C8 (no blue colonies were observed without adding SHP3-C8), and the intensity of blueness is dose-dependent of SHP3-C8 (0-600nM). This reporter tool is ready to be combined with random mutagenesis to seek genes playing a role in Rgg/SHP QS in *S. pneumoniae.*

MGCM PC 04

Investigating *Lactococcus lactis* response to phage infection at the proteome level

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The Gram-positive bacterium *Lactococcus lactis* is extensively used to produce an array of fermented dairy products. The phage sensitivity of industrial *L. lactis* strains poses a constant risk to milk fermentation processes despite the tremendous advancements in phage control in the last decades. Virulent lactococcal phages belonging to the sk1virus species (*Siphoviridae* family) are by far the most predominant in the dairy industry worldwide. Phage p2 is a model for this species and it infects *L. lactis* MG1363, the international reference strain for lactococcal research. Using mass spectrometry (MS)-based proteomics, we have generated comprehensive data on protein synthesis at various time points in a culture of *L. lactis*MG1363 before and during phage p2 infection. The MS approach resulted in the high-confidence detection and quantification of 56% of the theoretical bacterial proteome. We also identified proteins unique to infected bacteria and proteins with differing abundances during the infection. Using our high-throughput proteomic datasets to select the best gene candidates for inactivation is promising for the identification of host proteins involved in phage replication. We are currently using our recently adapted CRISPR-Cas9 tool to knock-out some of those bacterial genes. Additionally, we detected and quantified 78% of the theoretical phage proteome and identified many proteins of phage p2 that had never been detected so far. Amongst others, we uncovered a conserved small phage protein (ORFN1) coded by an unannotated gene. This work provides a unique view of the bacterial cells takeover by virulent phages and will provide novel information on phage-host interactions.

MGCM PC 05

Serum reduced media impacts on cell viability and cellular protein expression in human lung epithelial cells

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Transfection of human cells with RNA, DNA, plasmids or viral vectors is often used for research purposes. The companies selling the transfection reagents recommend to use serum-free or reduced serum median during the transfection. Opti-MEM medium has been used for more than a decade in many research studies for diluting the transfection reagents and sometimes as a transfection media. The media is also recommended for Mammalian Cell Culture. We are not aware of anyone reporting the impact of the Opti-MEM media on the expression of cellular proteins in human cells. In this study cell viability and expression of PSMA2, CLIC1 and HSPA5 in A549 cells were compared by immunoblotting after growing in Opti-MEM and DMEM medium. The transfection efficacy of the siRNA was measured in two different media targeting PSMA2 and CLIC1 genes. Cell viability was tested by WST-1 reagent and cellular morphology was monitored under the microscope at 200x magnification. Opti-MEM medium started negatively affecting the expression of PSMA2 and CLIC1, after 1 day of incubation but HSPA5 expression was increasing over time. Compared to DMEM, cell viability was very low in Opti-MEM and cellular morphology was affected by the media. Transfection in DMEM showed the similar efficacy of knockdown of the proteins without affecting the expression of the target proteins in the control. However, a widely-used loading control, GAPDH, was observed to decrease its expression in Opti-MEM. During an experiment, the adverse impact of media on cellular protein expression and cell viability can lead to miss-interpretation of the results. Careful choosing of loading control is also important, as Opti-MEM can dysregulate the expression of GAPDH, which was also found as an unstable reference marker in a colitis mouse model. DMEM media with nutrients and FBS was found promising media for transfection in A549 cells, as the cells were optimally growing in the media and expression of proteins were stable as tested with PSMA2, CLIC1, and HSPA5. The data clearly indicate that A549 cells are not happy in Opti-MEM. Consideration of these facts is necessary while using Opti-MEM as a culture or transfection medium. DMEM media with all nutrients and FBS could be an alternative option as a transfection media for A549 cells. The study was limited by testing only three proteins and only one cell type; thus, we cannot comment about the effects on other cellular proteins or in other cell lines at this point.

MGCM PC 06

MLJD1: a novel protein from *Enterococcus faecalis* involved in cell division

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We discovered a novel $DivIVA_{Ef}$interacting protein named MLJD1 (encoded by EF1025) which is conserved across Gram-positive bacteria with no homologues in Gram-negative bacteria. MLJD1 has a conserved putative DNA binding Helix-Turn-Helix domain at the C-terminal and two Cystathionine-Beta Synthase (CBS) domains at the central and N-terminal regions of the protein. The purpose of the present study was to ascertain that MLJD1 interacted with the cell division protein $DivIVA_{Ef}$. The size of MLJD1 was determined and its effects on cell division phenotype was ascertained by overexpression or inactivation *of mljd1* in *E. faecalis* was observed. We determined that MLJD1 interacts with $DivIVA_{Ef}$ using four methods– Yeast two Hybrid (Y2H), Bacterial two Hybrid), GST-pull down and Co-Immunoprecipitation. We also determined, by Y2H, that MLJD1 self-interacts and size exclusion chromatography indicated that it is a hexamer. *mljd1*was insertionally inactivated and this was lethal to*E. faecalis.* Cells were rescued by expression of *mljd1 in trans*. 10% of these cells exhibited long chain formation and abnormal cell division when viewed by light and electron microscopy. Extracellular vesicles, ranging between 20-300 nm in size and attached to the cell surface, were observed by electron microscopy in more than 50% *E. faecalis* cells overexpressing *mljd1*. Over and underexpression of the gene were confirmed by qPCR analysis. These

results indicate that MLJD1 is a novel DivIVA-interacting protein essential gene for cell division and viability in *E. faecalis*.

MGCM PC 07

Conjugative Complexities: Defining the requirements for transfer of *Rhizobium leguminosarum* plasmid pRleVF39b

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The genome of *Rhizobium leguminosarum* is compartmentalized into a chromosome and multiple large (> 150 kb) plasmids. Four conjugation systems (Types I to IV) have been identified that can mediate horizontal transfer of these plasmids. Type IV was initially identified in the Hynes lab on plasmid pRleVF39b. It is distinctive due to the presence of a truncated relaxase gene (*traA*) compared to the canonical relaxases, and a negative transcriptional regulator TrbR belonging to the Xre family, as well as the lack of common genes found in the other rhizobial conjugation systems. Annotated hypothetical proteins adjacent to known transfer genes in the type IV conjugation locus might have important functions in plasmid transfer. A series of unmarked deletions in each of the 14 hypothetical genes was created to identify essential and facilitating genes for the transfer of pRleVF39b. Marked mutants were created first by antibiotic cassette insertions and replaced by unmarked mutants to eliminate polar mutations. Each gene knockout was made in 2 strains: VF39SM and VF39SM cured of pRleVF39a (VF39a$^-$) to see if pRleVF39a, also self-transmissible, plays a facilitating role for pRleVF39b transfer. Finally, the effect of these mutations on the self-transfer of pRleVF39b from the mutant *Rhizobium* donors to plasmid free *Agrobacterium* recipients was analyzed. Among these mutations *orf23*, *orf24*, *orf26*, *orf27*, *orf28* and *orf29* mutants reduced the percent plasmid transfer frequency from *Rhizobium* donors to UBAPF2 recipients by 100-fold or more compared to the wild type (WT) suggesting their key role in conjugation. To determine the role of the TrbR regulator in pRleVF39b transfer, putative promoter (P) regions of *orf17*, *trbR*, *orf24*, *orf25*, *orf26* and *orf29* were fused to a promoter-less reporter gene *gusA*. The level of expression of promoters in WT VF39, VF39a-, VF39SM cured of pRleVF39b (VF39b-), *trbR* mutant and a *orf29* mutant was analyzed using β-glucuronidase assays. PtrbR expression was not reduced due to *trbR* mutation. Thus, TrbR does not auto-regulate itself. P24 and P25 showed a 400-fold increase in expression in VF39b- and *trbR* mutant backgrounds compared to WT, indicating that operons of *orf24* and *orf25* are part of the TrbR regulon. P24 and P25 expression was reduced in the *orf29* mutant background suggesting an activator role of Orf29. Expression of the *trbN* operon containing ATPase (*trbE*) and coupling protein (*traG*) genes, which are essential for conjugation, was tested under WT VF39, *trbR* mutant and *orf29* mutant backgrounds using qRT-PCR and the negative regulatory role of TrbR and the activator role of Orf29 was further confirmed. It was also identified that *orf16* and *orf17* are not part of the TrbRregulon and that *orf26* is not transcribed from a promoter immediately upstream of the gene.

MGCM PC 08

The effects of environmental and genetic factors on the germination of basidiospores in the *Cryptococcus gattii* species complex

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Natural and artificial hybridization has been frequently reported among divergent lineages within and between two closely related human pathogenic fungi *Cryptococcus gattii* species complex (CGSC) and *Cryptococcus neoformans* species complex (CNSC). However, the biological effects of such hybridization are not well known. In this study, we used CGSC and CNSC as models to investigate the potential effects of selected environmental and genetic factors on basidiospore germination of their mating crosses. Here we used five CNSC strains and twelve CGSC strains to create mating crosses to examine the effects of three temperatures (room temperature 23℃, optimum temperature 30℃ and mammalian body temperature 37℃), two media (a nutrient-rich YEPD medium and a nutrient-limited SD medium) and genetic divergence between pairs of parental strains on the germination rates of basidiospores. We found that the germination rates varied widely among crosses and environmental conditions, ranging from 0% to 98%. Overall, the two examined media showed relatively little difference on spore germination while the effect of temperature was notable, with the high temperature (37℃) having an overall deleterious effect on spore germination. Within CGSC, basidiospores from intra-lineage crosses generally had greater germination rates than those from inter-lineage and inter-species crosses, while environmental factors can significantly influence the pattern. Interestingly, progeny from some inter-specific crosses (CGSC x CNSC) showed germination rates comparable to some CGSC inter-lineage crosses. Our analyses indicate that all examined factors (temperature, medium, parental strain and strain pair) could influence basidiospore germination.