**Graduate Student Presentations**

***Chlamydia trachomatis*Manipulation of Protein Kinase C**

Prakash Sah, Ted Hackstadt , Erika Lutter

*Chlamydia trachomatis* is responsible for causing a range of diseases such as blinding trachoma and urogenital infections leading to serious complications. Inside a host cell, *C. trachomatis*lives in a parasitophorous vacuole called an inclusion from where it is able to secrete various effectors to manipulate host-cellular functions to its benefit. Currently, not much is known about Chlamydial manipulation of host kinases such as Protein Kinase C (PKC). PKCs are members of AGC family of kinases and involved in regulating various cellular functions such as, growth and proliferation, migration, survival and apoptosis. We hypothesize that *C. trachomatis* manipulates PKC pathways to regulate intracellular development inside the host, as PKCs are important in regulating various cellular functions. Indirect immunofluorescence of infected cells verified recruitment of multiple PKC isoenzymes to microdomains (Src-family kinases rich regions) on the inclusion. Recruitment of PKC substrates, including Marcks, was also confirmed.   Inhibition of PKC activity with Staurosporine at various time points resulted in decreased recoverable infectious progeny. These results confirm PKCs are important for intracellular growth and development of *C. trachomatis*.

**Regulation of the Phosphodiesterase RegA in *Dictyostelium discoideum* During cAMP Signaling.**

Nick Kuburich, Nirakar Adhikari, Jeff Hadwiger. Oklahoma State University

Many eukaryotic signaling pathways use cAMP as a secondary messenger to evoke specific responses to different external stimuli. Here, localized levels of cAMP can be controlled by phosphodiesterases, which are sometimes regulated by phosphorylation. *Dictyostelium discoideum* offers an excellent model system to study the regulation of phosphodiesterases as it contains relatively few cAMP-specific phosphodiesterases compared to mammals. The cAMP-specific phosphodiesterase, RegA, regulates important steps in *Dictyostelium* development and is negatively regulated by the MAP kinase, ERK2. This inactivation occurs periodically by external cAMP pluse where a cell-signaling pathway activates ERK2. Mammalian studies have suggested that the cAMP-dependent protein kinase, PKA, can also regulate the phosphodiesterase activity. This putative regulation of PKA on the activity of RegA has not been fully investigated in *Dictyostelium*. Mass spectrometry is being used to detect potential phosphorylation sites on RegA. Two sites of interest have been identified, including a PKA phosphorylation site. Site directed mutagenesis is being used to replace the residues at these sites and a MAPK site to mimic or prevent a phosphorylation event. The phenotypes of cells carrying these mutations will be analyzed through developmental analysis. RegA will be analyzed during multiple time points with phosphospecific antibodies to determine its regulation by kinases.

**Intracellular Calcium Regulates Antibiotic Resistance and Virulence in *Pseudomonas aeruginosa*.**

## Sharmily Khanam\*, Manita Guragain, Michelle King, and Marianna A. Patrauchan

Calcium (Ca2+) is a powerful secondary messenger in a human body. Therefore, imbalances in Ca2+ homeostasis are commonly associated with diseases, as exemplified by accumulation of Ca2+ in the pulmonary and nasal fluids of cystic fibrosis patients. Earlier, we established that *P. aeruginosa* maintains low intracellular Ca2+ level ([Ca2+]in), which transiently increases in response to extracellular Ca2+. We also identified several transporters responsible for maintaining Ca2+in homeostasis, disruption of which impaired multiple Ca2+-regulated traits, including antibiotic resistance and virulence factor production. Here we report identification of a putative Ca2+ channel, PA2604, that is required for the development of transient increases in [Ca2+]in and regulates Ca2+-induced tobramycin resistance. Genome-wide RNA-seq analysis revealed that PA2604 is involved in regulation of at least 342 genes in Ca2+-dependent manner. These genes include a number of virulence factors, known to be required for the development of the pathogen’s acute and chronic infections. Current research aims to validate selected RNA-seq data by monitoring promoter activities and to assess the relationship between PA2604 and Ca2+ responsive transcriptional regulators. The results provide the first experimental evidence of Ca2+in signaling in prokaryotes and shed light on the Ca2+in regulatory network controlling the virulence and antibiotic resistance of this pathogen.

**A Calmodulin-like Calcium Binding Protein, EfhP, Plays Role in Virulence of *Pseudomonas aeruginosa.***

Biraj B. Kayastha,Rendi Rogers, Mariette Barbier and Marianna Patrauchan

*Pseudomonas aeruginosa* is an opportunistic pathogen causing severe chronic infections in cystic fibrosis patients. Earlier, we have shown that its virulence is induced by Ca2+. We also reported a putative Ca2+-binding protein, EfhP, containing two predicted EF-hand motifs. We showed that EfhP mediates Ca2+ regulation of the pathogen’s infectivity and virulence factor production. Here, by using wax worm and murine macrophage infection model, we show that EfhP contributes to the pathogen’s virulence and intracellular survival. To confirm the ability of EfhP to bind Ca2+, we His-tag purified the soluble portion of the protein, and after removing the tag with TEV protease, subjected to Dynamic Light Scattering (DLS) and Isothermal Titration calorimetry (ITC). DLC indicated that EfhP forms dimers and tetramers. ITC confirmed that EfhP binds Ca2+ but not Mg2+. Currently, we aim to solve the multimeric state of the protein and calculate the Kd of Ca2+ binding. Further we aim to identify the residues involved in Ca2+ binding, by generating point mutations within the EF hands, and measuring Ca2+-binding. Future studies will aim to detect whether EfhP undergoes conformational changes upon Ca2+-binding, and identify its protein partners. Once confirmed, EfhP will be the first structurally characterized Ca2+ sensor in prokaryotes.

**LDH Gene Knockdown Using RNAi Techniques in the Anaerobic Fungus *Pecoramyces ruminantium* strain C1A.**

Shelby Calkins, Nicole Elledge, Stephen Marek, Mostafa Elshahed, Noha Youssef.

Members of the anaerobic gut fungi (AGF) reside in rumen, hindgut, and feces of ruminant and non-ruminant herbivorous mammals and reptilian herbivores. They constitute a basal fungal lineage with an asexual life cycle involving a swimming zoospore stage that usually germinates into a vegetative hyphal stage upon exposure to a solid surface or a carbon source. Our recent efforts have not only produced the first published genome of an AGF isolate (*Orpinomyces* sp. strain C1A), but also produced the first anaerobic flooding technique, which allows for the collection of germinating zoospores. Currently, there is not a genetic system available for AGF, as they tend to be difficult to maintain in a laboratory setting due to their strict anaerobic nature. Thus, establishing a gene manipulation technique such as RNA interference (RNAi) would be useful for studying gene function. It has been shown previously in other filamentous fungi, e.g. *Aspergillus* species, that germinating spores can uptake short interfering RNA (siRNA), and were thus used for RNAi-mediated gene silencing approaches to successfully knock down the function of a particular gene of interest. Therefore, and as a proof of principal, we attempted RNAi using the established RNAi-mediated gene silencing technique in order to knock down the function of the D-lactate dehydrogenase (D-*LDH*) gene in the AGF *Pecoramyces ruminantium* strainC1A (C1A). We chose *LDH* because it is a single copy gene in the C1A genome, and inhibition of LDH theoretically should not affect the survival of this fungus. We hypothesize that 1) RNAi techniques using synthetic siRNAs can be successfully applied in AGF systems (e.g. strain C1A), and that 2) the D-*LDH* gene can be knocked down using RNAi established technique, resulting in a detectable decrease in D-lactate production in the growth medium. Results from experiments on multiple biological replicates are promising at the RNA and protein level, where levels of D-lactate in siRNA-treated samples were significantly lower than in the wild type. To our knowledge, this is the first successful gene manipulation attempt in anaerobic fungi that will potentially open the door for more gene function studies in this understudied fungal clade.

**Comparative Phylogeny of the NDH-1 Complexes of Cyanobacteria**

N. Miller, J. Artier, and R. Burnap

NDH-1 Complexes are a vital part of cellular respiration, with homologues found in all domains of life. They receive electrons from a reductant pool and utilize the redox energy to pump protons across a membrane. The generated proton motive force is then used to synthesize ATP. Cyanobacteria utilize these complexes in a unique way. Throughout their evolution subunits were copied and changed, providing the ability for CO2 hydration to HCO3-, allowing the accumulation of inorganic carbon inside the cell membrane to compensate for Rubisco’s poor affinity for CO2. While the functions of the complexes have been studied extensively, the importance of individual subunits’ amino acid constituents has not been explored. By comparing the sequences and putative structures of the CO2 hydrating subunits to well-studied NDH-1 complexes from *Escherichia coli* and *Thermus thermophilus*, structure and function of the Cyanobacterial NDH-1 complexes may be explored. A mutant deficient in CO2 hydrating NDH-1 complexes and several HCO3- transporters has been constructed. It will be used as a recipient strain for point mutations in future work, and as a base comparison point to the wild type and complement in this study. Photosynthetic physiology measurements of photosynthetic efficiency and Ci affinity will be used to characterize the wild type, deletion mutant, complement, and, in the future, point mutants of subunits of the CO2 hydrating NDH-1 complex.

**Global distribution patterns and pangenomic diversity of the candidate phylum "Latescibacteria" (WS3)**

Ibrahim F. Farag, Noha H. Youssef, and Mostafa S. Elshahed\*

We investigated the global distribution patterns and pangenomic diversity of the candidate phylum *“*Latescibacteria” (WS3) in 16S rRNA gene as well as metagenomic datasets. We document distinct distribution patterns for various “Latescibacteria” orders in 16S rRNA gene datasets, with prevalence of orders sediment\_1 in terrestrial, PBSIII\_9 in groundwater and temperate freshwater, and GN03 in pelagic marine, saline-hypersaline, and wastewater habitats. Using a fragment recruitment approach, we identified 68.9 Mb of “Latescibacteria”-affiliated contigs in publicly available metagenomic datasets comprising 73,079 proteins. Metabolic reconstruction suggests a prevalent saprophytic lifestyle in all “Latescibacteria” orders, with marked capacities for the degradation of proteins, lipids, and polysaccharides predominant in plant, bacterial, fungal/crustacean, and eukaryotic algal cell walls. As well, extensive transport and central metabolic pathways for the metabolism of imported monomers were identified. Interestingly, genes and domains suggestive of the production of a cellulosome, e.g. protein-coding genes harboring dockerin I domains attached to a glycosyl hydrolase, and scaffoldin-encoding genes harboring cohesin I and CBM37 domains, were identified in orders PBSIII\_9, GN03, and MSB-4E2 fragments recovered from four anoxic aquatic habitats; hence extending the cellulosomal production capabilities in Bacteria beyond the Gram-positive Firmicutes. In addition to fermentative pathways, a complete electron transport chain with terminal cytochrome C oxidases Caa3 (for operation under high oxygen tension), and Cbb3 (for operation under low oxygen tension) were identified in PBSIII\_9, and GN03 fragments recovered from oxygenated, and partially/seasonally oxygenated aquatic habitats. Our metagenomic recruitment effort hence represents a comprehensive pangenomic view of this yet-uncultured phylum, and provides broader and complimentary insights to those gained from genome recovery initiatives focusing on a single or few sampled environments.

**Anaerobic fungal diversity: Is the sky really the limit? Re-evaluating the taxonomic positions of different lineages within the phylum using culture and molecular based approaches**

Radwa A. Hanafy, Britny Johnson, Mostafa S. Elshahed, Noha H.Youssef

The Anaerobic gut fungi (phylum *Neocallimastigomycota*) reside in the gastrointestinal tract of mammalian and non-mammalian herbivores, and represent one of the early-diverging fungal phyla. They are characterized by their broad fermentative capabilities, strict anaerobic physiology, extremely low G+C content, and the presence of a flagellated zoospore stage in their life cycle. Eight genera are currently affiliated with the phylum. As a part of a wider effort to resolve the evolutionary history of the phylum and genera within, we are currently isolating, characterizing, and sequencing the genomes of representatives belonging to each of the known AGF genera. Isolation efforts from the feces of cow, goat, and sheep yielded several isolates (n=51). The genomes of five different isolates and transcriptomes of 17 of the current isolates were sequenced. Classification of the current isolates, using microscopic and phylogenetic-based approaches, identified members of the genera *Anaeromyces*, *Neocallimastix*, *Orpinomyces*, *Piromyces*, and *Caecomyces.* Interestingly, among the 51 obtained isolates, 11 isolates belonged to a novel genus, *Pecoramyces,* that is phylogenetically closely related to members of the *Orpinomyces* genus but morphologically distinct based on its monocentric thallus and monoflagellated zoospore. Also, *Anaeromyces* isolates exhibited distinct morphological features, including entangled hyphae and intercalary sporangia, which have not been previously reported in *Anaeromyces* species. Here we present a detailed description of the novel genus *Pecoramyces,* and a comprehensive definition of the anaerobic fungal genus *Anaeromyces* boundaries using both culture and sequence data analysis based approaches. Future work will involve genomic and transcriptomic analyses aimed towards resolving the evolutionary history of the phylum *Neocallimastigomycota* and genera within in the fungal tree of life, and correlating the timing of such phylum/genera evolution events to the evolution of their host.

**Detection and Characterization of Antibiotic Resistant *S. aureus* from Cystic Fibrosis Patient Isolates**

Rawan G. Eleshy1, Nighat F. Mehdi2, and Erika I. Lutter1

Cystic fibrosis (CF) is a common genetic disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator gene (CFTR). Mutations within this gene inhibit the function of the chloride ion channels across epithelial membranes. This leads to the formation of thick mucus within the lung airways of CF patients. Therefore, the CF lung becomes an excellent environment for bacterial colonization. *S. aureus* is the first pathogen to colonize the lungs and tends to persist throughout the lives of CF patients. *S. aureus* is known for its ability to develop resistance against antibiotics. Antibiotic resistance is one of the biggest problems faced in medicine today. This study aims to detect and characterize the resistance of *S. aureus* obtained from CF patients of various age groups to a panel of clinically relevant antibiotics. Based on findings from previous studies, there are nine antibiotic resistance genes in *S. aureus* that have been correlated with CF patients. Using PCR amplification, we checked if any of these resistance genes are present in the CF isolates. In addition, we performed antibiotic susceptibility tests to determine if these isolates exhibit a resistant phenotype. Minimum inhibitory concentrations (MIC’s) of each antibiotic to each isolate were determined to further confirm resistance. In conclusion, the presence of resistance genes and susceptibility to antibiotics differ among CF patients. CF isolates showed both susceptibility and resistance to the tested antibiotics, but the percentage of resistant isolates was higher. The interesting finding was that resistance to antibiotics, in some isolates, did not correlate with the presence of resistance genes. The lack of resistance genes in isolates that showed a resistant phenotype to antibiotics suggests that *S. aureus* is using other mechanisms to acquire resistance. This study shines the light on understanding *S. aureus* as a CF pathogen and its resistance within the CF lung. This will aid in enhancing treatment options for CF patients to help them live longer and more productive lives.

**A β Propeller Protein, CarP, Plays Role in *Pseudomonas aeruginosa* Response to Calcium**

Michelle King**,** Mariette Barbier,and Marianna A. Patrauchan.

*Pseudomonas aeruginosa* is an opportunistic pathogen that causes severe acute and chronic infections in humans, particularly, in cystic fibrosis (CF) patients. Our group has shown that calcium (Ca2+) induces virulence and antibiotic resistance in *P. aeruginosa*. Earlier we identified a Ca2+-regulated protein, CarP, which was predicted to form a 5 bladed β-propeller structure with a putative Ca2+ binding site in the center of the propeller. We characterized its role in several virulence-related Ca2+-dependent phenotypes and cell tolerance to high Ca2+. To further characterize the role of CarP in Ca2+-regulated virulence and adaptation to host, we aim to identify the host factors that control the expression of *carP.* For this,we constructed a reporter with *carP* promoter cloned upstream of the *lux* operon, which allows measuring the promoter activity.. In addition to elevated Ca2+, CO2 and oxidative stressor, H2O2, we will test the effect of antibiotics used to treat *Pseudomonas* infections. Furthermore, we investigated the role of CarP in virulence by using *Galleria mellonella* and mouse virulence models. Disruption of *carP* reduced worm killing by 60% and decreased survival of *P. aeruginosa* in mice by 30%. This data reveals that CarP plays an important role in the pathogen’s virulence and survival within a host. Further studies aim to characterize Ca2+-binding capabilities of CarP and advance our knowledge on the molecular mechanisms of Ca2+ regulation of *P. aeruginosa* virulence and fitness in response to host environment*.*

**Undergraduate Student Presentations**

**Allelic Exchange Mutagenesis of *Chlamydia trachomatis***

Jordan Fleming and Erika Lutter

*Chlamydia trachomatis* is a well-known sexually transmitted infection that infects millions of people annually around the world with over 30 million new cases occurring within the United States alone. *C. trachomatis* is an obligate intracellular pathogen and has the ability to take up many of the host's assets in order to survive and proliferate. During intracellular survival, *C. trachomatis* resides inside of a parasitophorous vacuole, termed an inclusion, which is decorated with Chlamydial proteins called inclusion membrane proteins. These proteins are predicted to be responsible for various host interactions and the recruitment of host proteins to the inclusion for Chlamydial use. With the advent of novel genetic tools it is now possible to generate mutations in the chlamydial genome of specific genes. This project focuses on the preparation of fluorescence-reported allelic exchange mutagenesis constructs targeting the inclusion membrane proteins in the CT229-224 operon. The first two genes in the operon have known functions but CT227-224 do not. Targeted genomic deletion of each of the remaining genes in the operon will provide insights into the role of each gene in chlamydial development and growth.

**Comparative genomic analysis of the Neocallimastigomycota CAZyome**

Chelsea Murphy

Members of the anaerobic fungi (Neocallmastigomycota) are known for their ability to degrade plant polysaccharides in the anoxic herbivorous gut. We aim to understand the evolutionary history, co-occurrence patterns and pangenomic diversity of the Carbohydrate Active Enzymes (CAZymes) repertoire in the Neocallimastigomycota. To this end, we sequenced the transcriptomes of fourteen different Neocallimastigomycota species. Those, along with four previously available transcriptomes, represent six different Neocallimastigomycota genera. From the transcriptomes, we extracted the protein families (pfam) domains encoding the glycoside hydrolases and polysaccharide lyases and analyzed the patterns of occurrence and phylogenetic diversity of these modules. The Neocallimastigomycota panCAZyome consisted of 128 families out of the 136 currently recognized, with the notable absence of the GH7 and GH61, both of which are ubiquitous in other fungal lineages. Interestingly, in spite of the large number of transcripts (465 to 1814) identified per transcriptome, it appears that all GH families consist of 1-5 distinct orthologues, suggesting massive gene duplication in the Neocallimastigomycota genomes. Further, within these orthologues, the majority appears to be obtained from bacterial donors by horizontal gene transfer, attesting to the important of this process in conferring plant biomass degradation capacity and ecological fitness to this group of fungi. Most HGT events were observed in transcriptomes of all genera, suggesting the occurrence of this process prior to genus level diversification within this lineage. Major bacterial donors include lineages known to be prevalent in the herbivorous gut, e.g. Clostridiales, Fibrobacteriales, and Ruminoccoccales, although additional events from lineages not associated with the herbivores guts were identified. Collectively, this work demonstrates the ancient origin and massive occurrence of HGT as a driving force behind the observed AGF efficient plant biomass degradation abilities.

**Recruitment of Protein Kinase C and Protein Kinase C substrates to the *Chlamydia trachomatis* inclusion**

**Nick Nelson, Prakash Sah, and Erika Lutter**

*Chlamydia trachomatis* is an intracellular pathogen that causes roughly 3 million infections each year in the United States. Infections caused by *C.trachomatis* include ocular and urogenital infections that can lead to further complications such as infertility and ectopic pregnancy. During infection *C. trachomatis* lives in a parasitophorous vacuole termed an inclusion. From within the inclusion the bacteria manipulate host-cell functions in order to aid in its survival. Many proteins are recruited to this inclusion including Protein Kinase C (PKC). PKC is a member of the AGC family of kinases ,which has been shown to be involved in various cellular functions such as apoptosis, proliferation, migration, growth, and survival. We hypothesize that these Kinases are recruited the inclusion membrane of *C. trachomatis* to aid in its survival. Within this study we investigate the recruitment of PKC’s (gamma and delta isoenzymes) and one of their substrates, CPI-17. A Eukaryotic expression plasmid with a fluorescent tag (mCherry and eGFP) were generated and transfected into infected HeLa cells to investigate recruitment to the inclusion during active infection. Verifying utilization of PKC and PKC substrates during infection by *C. trachomatis* is essential to deciphering host-pathogen interactions and may lead to future novel therapeutic targets.

**Regulation of the cold shock response in *Escherichia coli***

Amanda Demackiewicz, Jerreme Jackson, Tyrrell Conway

Sudden shifts above or below optimal growth temperatures elicit physiological responses in microorganisms that allow them to survive. With direct implications in food safety and sustainability, the cold shock response in *Escherichia coli* has been studied extensively. Cold shocked *E. coli* experience a rapid reduction in translation efficiency by ribosomes and the consequential reduction in protein synthesis. Also observed are concomitant changes in membrane fatty acid composition, to help maintain a level of fluidity that permits normal function, and the induction of cold-inducible proteins, which aid in the acclimation to the temporal environmental conditions. While a vast amount of research has keyed on the immediate response to a rapid temperature downshift, the simultaneous regulation of cold-inducible and general stress response signaling is not completely understood. This work focuses on characterizing the role of cold shock proteins (cspA-I) following a temperature downshift from 37°C to 12.5°C. Using reverse transcriptase polymerase chain reaction (RT-PCR), we analyzed gene expression during exponential growth, cold shock-induced growth arrest, and the second exponential phase of growth in WT and RpoS mutant (∆RpoS) *E. coli*. .

**Can enrichment conditions be tailored for targeting various genera of anaerobic gut fungi (*Neocallimastigomycota*) or is it completely random?**

Britny Johnson, Radwa A. Hanafy, Mostafa S. Elshahed, Noha H.Youssef

Anaerobic gut fungi, those residing in the gut of herbivorous mammals and reptiles, belong to a single phylum, the *Neocallimastigomycota*. This phylum has a basal position in the fungal tree of life and all its members are strictly anaerobic. They play a pivotal role in the animal digestion by helping break down complex plant fibers into simple sugars and short chain fatty acids that the animal uses for energy. They are capable of this role via an arsenal of glycosyl hydrolases, carboxyl esterases, and polysaccharide lyases. As part of a wider effort geared towards studying the phylogenetic position of the phylum, our lab set on a journey to obtain representative isolates to cover all known cultured genera of the phylum. During this 18-month long journey, we obtained 51 isolates representing 6 of the genera (total number of known genera so far is 9). The goal of this study is to investigate the role played by different factors, namely the carbon source, the host, and the type and age of the sample, on the identity of the isolates obtained. In other words, can we predict based on the above factors used during isolation which genus will be selected for, or is the process completely random? Work is underway to statistically identify the significant factors, if any, and devise a simple model for predicting the genus identity. Future work will include testing our model on new samples.

**Antimicrobial Properties of Novel Silver (I) Cyanoximates**

Erin Gallaway, Treyon Grant, Snow Popis, Nikolay Gerasimchuk, and Marianna A. Patrauchan

Biofilms are microbial communities that grow on surfaces and are embedded into extracellular polymeric matrices, which consist of polysaccharides, proteins, and extracellular DNA. Biofilms induce multiple virulence factors and horizontal gene transfer making them more resistant to antimicrobials and host factors. The ability to form a biofilm plays a major role in the development of infections. The increase in microbial resistance introduces an important clinical challenge, particularly in cases associated with implants, which have a high predisposition for developing infections. This requires the development of alternative antimicrobial practices to prevent bacterial infections. We have synthesized a series of novel silver(I) cyanoximates that have remarkable resistance to high intensity visible light, UV, and heat with a broad range of water solubility. The goal is to characterize the antimicrobial properties of these compounds and test if they have a potential as antimicrobial additives to implant materials. We have incorporated these compounds into polymeric composites, and tested their antimicrobial activities against both planktonic and biofilm growth of several diverse human pathogens, such as *Pseudomonas* *aeruginosa,* the most frequent Gram negative agent infecting implants, and *Staphylococcus aureus,* which ishighly resistant Gram positive pathogen. Biofilm quantification by crystal violet biofilm assay followed by scanning electron microscopy confirmed the high antimicrobial potential of the compounds, particularly AgPiCO yellow and AgPiCO red. Currently we are testing possible synergistic effect of the compounds with tobramycin and trimethoprim, the antibiotics commonly used to treat *P. aeruginosa*and *S. aureus*infections.

**Calcium Binding in the EF-Hand Protein, EfhP, Regulating Calcium-Dependent Virulence in *Pseudomonas aeruginosa***

Rendi Rogers, Biraj Kayastha, Marianna A. Patrauchan

*Pseudomonas aeruginosa* is a human pathogen that, along with causing other various types of infections, is the leading cause of death in patients with cystic fibrosis. Research in our lab has shown that calcium (Ca2+) induces virulence in *P. aeruginosa.* Aiming to identify the main components of Ca2+ signaling and regulatory networks in *P. aeruginosa,* our lab predicted several putative Ca2+-binding proteins and characterized their role in *P. aeruginosa* virulence. One of them is EfhP protein, containing two canonical EF-hand domains. The EF-hand motif has been studied in eukaryotes and is shown to bind Ca2+. We hypothesize that EfhP binds Ca2+ and plays role in *P. aeruginosa* Ca2+-induced virulence. In order to characterize Ca2+-binding properties of EfhP, the encoding gene (PA4107) was successfully cloned into the vector pSKB3 and expressed in *E. coli*. Then, the protein was purified by affinity chromatography. Current studies aim to assess the Ca2+ binding capabilities and specificity of EfhP by using isothermal titration calorimetry (ITC). A Ca2+-binding constant will be calculated, and the Ca2+ binding affinity will be compared to that of Mg2+. This information is expected to confirm the role of EfhP as a molecular component of Ca2+-signaling. Further, the amino acid residues predicted to bind Ca2+ will be identified by characterizing Ca2+-binding properties of point mutated EfhP. Finally, the conformational changes occurring in EfhP upon binding Ca2+ will be measured.

**Elevated levels of Calcium increases rhamnolipid production in *Pseudomonas aeruginosa***

Mandy Truelock, Michelle King, Mariana Patrauchan

*Pseudomonas aeruginosa* is a gram-negative, opportunistic pathogen known to infect open wounds, burns, and the lungs of Cystic Fibrosis patients. Calcium (Ca2+) has been known to induce virulence factors of *P. aeruginosa* such as pyocyanin production and swarming motility, which has been shown to be required for biofilm formation. In order to swarm across semi-solid surfaces *P. aeruginosa* secretes a biosurfactant called rhamnolipid. In addition, rhamnolipid is a virulence factor that aids in defense against the host immune response. Based on the observation that swarming motility is induced by Ca2+, we hypothesize that rhamnolipid production is also increased in the presence of Ca2+. In order to demonstrate this, we are testing the effect of elevated Ca2+ on the expression of *rhlA*, the gene required for rhamnolipid production. The next aim will be to test the role of several earlier identified Ca2+-binding proteins (EfhP, CarP, and PA2604) in regulating Ca2+-induced rhamnolipid production. The mutants with each of the corresponding genes disrupted showed significant alterations in Ca2+-induced swarming. We will transform these mutants with the *rhla*-gfp fusion containing plasmid and monitor fluorescence during growth at varying Ca2+ concentrations. We anticipate that these mutants will also show a reduction in Ca2+-dependent rhamnolipid production.

**Generating mutations for functional studies of the putative Ca2+-binding protein CarP.**

Daniel McLeod, Michelle King, Sharmily Khanam, Marianna Patrauchan

*Pseudomonas aeruginosa* is an opportunistic pathogen that infects the lungs of cystic fibrosis patients and wounds from surgery or burns. Previously, we found that several virulence factors of *P. aeruginosa* are induced by calcium (Ca2+). We identified a hypothetical periplasmic protein, CarP, which has been shown to play a role in several Ca2+-induced virulence factors. Therefore, we hypothesize that this protein plays a role in *P. aeruginosa* Ca2+ regulatory network. To study the role of CarP in Ca2+-dependent phenotypes we used a complementation strain *carP*::Tn5/*carP,* where *carP* is cloned under an arabinose inducible promoter. Our first goal is to generate an alternative complemented strain that would not require addition of arabinose or any other inducers. Our second goal is to study Ca2+ binding of CarP and identify the amino acids that are responsible. To test this prediction, we will make point mutations replacing each amino acid predicted to bind Ca2+ with glutamine, purify the protein and measure its ability to bind Ca2+. Considering the lack of similarity of CarP with characterized Ca2+-binding proteins, we anticipate to identify a novel Ca2+ binding motif. Obtaining these mutants will also enable future functional studies, characterizing the role of CarP in *P. aeruginosa* virulence.

**Antibiotic Resistance of *Pseudomonas aeruginosa* Recovered From Cystic Fibrosis Patients**

William Starr (Undergraduate), Rawan Eleshy, Nighat Mehdi, and Erika Lutter

Cystic Fibrous (CF) patients produce dehydrated thick mucus in their lungs and lack the ability to clear this mucus due to mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR gene). Once the infection has been acquired, eradication of P. aeruginosa from the CF lung is rare. This study aims to determine resistance profiles of *P. aeruginosa* clinical isolates. Kirby-Bauer tests were performed on 52 isolates using nine different antibiotics which represent multiple antibiotic classes. In addition, DNA was extracted from the CF isolates and PCR was performed to verify the presence of eight prominent antibiotic resistance genes. The results showed that all of the isolates had resistance to at least one of the nine antibiotics; however, not all of isolates showed the presence antibiotic resistance genes by PCR. Results indicated that higher dosing of antibiotics is needed for CF patients due to infections being able to survive the immune system, smaller antibiotic treatments, and swapping of genetic material between bacterial species. By understanding antibiotic resistance of *P. aeruginosa* from CF patients in regards to the mechanisms in which this resistance is acquired, treatment options for CF patients can be more specialized.