Relationship between two signaling systems: Calcium and Cyclic nucleotide

Pseudomonas aeruginosa is an opportunistic human pathogen causing severe acute and chronic infections, which are difficult to treat because of the pathogen's extreme antibiotic resistance. It is a leading cause of hospital acquired infections and death of cystic fibrosis (CF) patients. Calcium (Ca^{2+}) is a well-known signaling molecule in eukaryotes, and its levels in human body are imbalanced in response to infection or disease. Such Ca^{2+} imbalances may be sensed by bacterial pathogens and trigger infections. Our lab has previously shown that Ca^{2+} enhances the ability of *P. aeruginosa* to cause a disease. **However, the mechanisms of Ca^{2+} induction of *P. aeruginosa* virulence are yet to be understood.** Our lab also showed that *P. aeruginosa* maintains low basal level of free intracellular Ca^{2+} ($[Ca^{2+}]_{in}$) and responds to external Ca^{2+} by transiently increasing $[Ca^{2+}]_{in}$. This suggests the signaling role of $Ca^{2+}{}_{in}$. However, there is no direct experimental evidence supporting it. **Therefore, the first goal of my research is to provide a direct experimental proof of the signaling role of $[Ca^{2+}]_{in}$ in *P. aeruginosa*.**

We have predicted five genes responsible for producing transient changes in $[Ca^{2+}]_{in}$ in *P. aeruginosa* cells in response to external Ca^{2+}. In the previous semester, we measured $[Ca^{2+}]_{in}$ in the transposon mutants with the corresponding gene disruptions and identified a Ca^{2+} "blind" strain, which lacks functional PA2604 and is defective in producing $[Ca^{2+}]_{in}$ transients. This mutant will be used as a **tool** to experimentally prove the signaling role of $[Ca^{2+}]_{in}$. Recently, I tested the role of $[Ca^{2+}]_{in}$ signaling in regulating Ca^{2+} induced phenotypes. The results of my experiments showed that the Ca^{2+} blind mutant was unable to produce calcium induced swarming motility as compared to the wild type (WT) PAO1. This semester I propose to determine whether the presence of Ca^{2+} (3-5 mM) or its absence (chelated by 3-5 mM EGTA) would affect the mutant's growth in both liquid medium and on a surface as a biofilm. Studying biofilm formation is highly important, as *P. aeruginosa* forms biofilms while infecting a host. To characterize biofilm growth I will learn and use confocal microscopy (available at the OSU core facility). We predict that the "blind" mutant will not respond to Ca^{2+} in the medium. This will confirm the role of Ca^{2+} signaling in regulating Ca^{2+}-dependent processes.

My second proposed aim is to determine the role of $[Ca^{2+}]_{in}$ homeostasis in cyclic nucleotides signaling. Cyclic nucleotides such as cyclic adenosine monophosphate (cAMP) and cyclic di-guanosine monophosphate (c-di-GMP) are well-established intracellular signaling molecules. Both cyclic nucleotides and Ca^{2+} regulates physiology and virulence in *P. aeruginosa*. **However, the relationship between the two signaling systems is not known.**

For this aim, I will measure the effect of Ca^{2+} on the ability of the WT and Ca^{2+} "blind" mutant to produce and accumulate cAMP and c-di-GMP. I will learn and use high performance liquid chromatography (HPLC) approach, which has been successfully used by my graduate mentor in the lab. I will use the HPLC instrument available at the OSU core facility. We expect that the WT PAO1 will show Ca^{2+}-regulated production of c-nucleotides, however the "blind" mutant will show no response to external Ca^{2+}.

This research is very important, as it will provide the experimental evidence supporting Ca^{2+} signaling and its regulatory power in a human pathogen *P. aeruginosa*. Overall, establishing the signaling role of intracellular Ca^{2+} will improve scientific understanding of the molecular mechanisms involved in regulation of virulence in *P. aeruginosa*. These experiments will generate a new knowledge, which will also help understanding the interactions between the pathogen and host environment with elevated Ca^{2+} levels. Therefore these results of this research will ultimately provide scientists with novel targets that can be used to develop new drugs for combating *P. aeruginosa* infections. This research project is very exciting for me, as it will give me valuable exposure to research process and provide me with the opportunity to gain essential critical thinking skills. In addition, this research will allow me to learn about the pathogen that has a significant impact on humans. The knowledge I gain will help me understand how pathogens cause disease, and what strategies can be considered for successful control and management of infectious disease. The project will give me a start to hands-on research, which will be crucial, as I want to continue pursuing a career in the field of medical research. So, the Wentz scholarship will not only allow me to be a part of a group solving a challenging scientific question, but also let me contribute new data to medical science, and therefore will make my education at OSU so much more exciting, meaningful, and fulfilling.

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