Joseph Paquette

Honors Project Description

Assay for the Presence of PriM protein in Outer Membrane Vesicles in LVS *Francisella tularensis*

As my Honors Project at the University of Rhode Island, I will be working with Dr. Kathryn Ramsey of the Cell and Molecular Biology Department in the College of the Environment and Life Sciences and the Biomedical and Pharmaceutical Sciences Department in the College of Pharmacy. Dr. Ramsey's laboratory studies the human bacterial pathogen *Francisella tularensis*, with a strong focus on its pathogenesis as well as the fundamental genetic properties which are responsible for its virulence. The laboratory uses a Live Vaccine Strain (LVS) of *F. tularensis*, which is an attenuated version of the organism that allows it to be used safely for research purposes. One of the major projects currently in motion in Dr. Ramsey's lab is the study of the protein PriM, which when expressed in large amounts contributes to a significant decrease in virulence, quantified specifically by a decrease in intramacrophage survival. In an attenuated, but genetically similar subspecies of *Francisella*, *F. novicida*, the PriM protein has been shown to be present in outer-membrane vesicles (OMV). When intact these OMV have also been previously shown to trigger host cell inflammatory responses, which is a reasonable hypothesis as to why *F. novicida* is not pathogenic to humans. However, it has not been confirmed whether or not the PriM protein is present in these same OMV in *Francisella tularensis*. For my Honors Project I am proposing that I use available methods of OMV purification and protein analysis to determine whether PriM is a component of OMVs in *Francisella tularensis*. Under standard conditions in wild-type *F. tularensis*, the gene which encodes PriM is repressed by the protein PmrA.

Therefore, we will test for the presence of PriM in OMV using both wild-type cells and cells in which the gene for PmrA has been deleted (Δ*pmrA*). By the end of this project, I would like to have completed the following objectives: Successfully purify OMV from *F. tularensis*; learn the proper procedure and techniques for verifying the presence of a protein, such as a Western blot; and ultimately confirm or deny the presence of PriM in OMV in *F. tularensis*.

Any and all materials needed to complete the objectives outlined above will be initially available in Dr. Ramsey's laboratory, but additional materials will need to be purchased. Therefore, I will apply for a grant from the Honors Program. These materials include but are not limited to test tubes, microcentrifuge tubes, protein analysis and purification reagents, and culture media.

From September of 2018 to May of 2019, I worked as an undergraduate research assistant in Dr. Ramsey's laboratory, and I will be returning for the spring semester of 2020 to complete my Honors project. In Dr. Ramsey's lab, I learned various basic techniques of microbiological and biochemical research. These techniques included preparation and conduction of polymerase chain reactions, creation and transformation of plasmids, purification of genomic DNA, preparation of growth media, plating techniques, as well as electroporation and allelic exchange. I have also taken the Advanced Microbiology course and laboratory here at URI, where I performed dilution series, extraction of plasmid DNA via MiniPrep, and ß-galactosidase assays. Each of my experiences in Dr. Ramsey's lab as well as the material I have learned in such courses as Biochemistry, Microbiology, Genetics, and Pathogenic Bacteriology will serve me well in order to properly conduct the necessary experiments and procedures to effectively test my hypothesis regarding PriM in outer membrane vesicles in LVS *F. tularensis.* Aside from the hands-on techniques I have learned in the laboratory, my time working with Dr. Ramsey and her

graduate students has further developed the ways in which I approach scientific research. After nearly a year in a research environment, I have expanded my familiarity with scientific language, which has in many ways contributed to enhancing my understanding of the information and concepts which I am studying. The way in which I approach the research process has evolved, largely in part to observing the techniques of different graduate students and professors. I consistently attempt to view projects on a holistic scale as to see the big picture, because it is very easy to get lost in the small, complicated details of an experiment.

Ultimately, I plan to attend graduate school to earn a PhD in molecular biology and/or microbiology. The research projects of which I have been a part in the past as well as the ones in which I will participate in the future will be of great value to me when I am in the process of earning this advanced degree. I am very grateful to have the opportunity to engage in projects of scientific discovery as an undergraduate student at the University of Rhode Island. Each day I spend in the laboratory with Dr. Ramsey further enhances my chances of success in both the completion of my Honors project as well as in my future endeavors in the field as a molecular biologist.