Date: May 7, 2024

Project Title: Assessing the contribution of a cell wall enzyme to the survival of *F. tularensis* in freshwater

Primary Student Name: Johanyx Rodriguez

Project start date: December 1, 2023 **Project end date**: July 25, 2024

Faculty Mentor Name: Kathryn Ramsey

Department: Cell and Molecular Biology

Abstract:

Francisella tularensis is a Gram-negative pathogen and is the causative agent of the infectious disease tularemia. Though *F. tularensis* has been found to survive in freshwater for a long period of time, how the bacterium survives in freshwater is still understood. The overarching goal of this research project is to advance our understanding of the bacterium and its survival mechanisms in freshwater environments. My goal was to determine if the enzyme that is encoded by the *mpl* gene is necessary for the survival of *F. tularensis* in freshwater. I successfully cloned a plasmid that will allow us to modify the F. tularensis LVS genome to remove the *mpl* gene. We are currently in the process of making the mutant without the mpl gene. Currently, I have been piloting the freshwater survival assay while replicating previous findings preparation for once we obtain the mutant. I will use these freshwater survival assays to assess if the *mpl* gene is important for *F. tularensis* freshwater survival.

Outcomes

In this project, I was able to successfully clone the plasmid pKR200 which will be used to modify the *F. tularensis genome*. To do this, I started by amplifying two pieces of genomic DNA that flank either side of the *mpl* gene. I purified these DNA fragments and the plasmid backbone. I ligated the DNA inserts onto the plasmid backbone and transformed *E. coli*. I then grew the bacteria on LB plates with the antibiotic kanamycin. Cells that contain *kanR* (a gene encoding kanamycin-resistance that is part of the plasmid backbone) grew on this media have the pKR200 plasmid. Through sequencing I was able to validate that the plasmid sequence was correct.

Once I successfully cloned the plasmid, I started the protocol for allelic exchange using this plasmid to create cells lacking the *mpl* gene. With my graduate student mentor, we have successfully completed multiple steps of the protocol, but have not yet obtained the desired mutant for technical reasons. Starting May 20, I received an opportunity from the Science &

Engineering Fellowship where I will be continuing the my project over the next 10 weeks. I expect to use my increased technical skills to successfully obtain a mutant in the near future.

When we eventually achieve the last few steps of allelic exchange, we will then move onto the final steps of the project which is a freshwater survival assay. During the past few weeks, I have been practicing for the freshwater survival assays where I have been replicating data a previous undergraduate student has generated. While replicating this data I have learned new skills such as conducting serial dilutions, data analysis as well as the plating and counting of cells using a wild-type strain and a mutant strain that possibly would not survive in freshwater. I had setup 6 flasks that contain 4-degree Celsius freshwater from a local river. This was a mock environment to grow the mutant cells and our negative control group that contain *F. tularensis LVS*. Over a period 4 weeks, I had conducted serial dilutions and plate these diluted cells to eventually calculate cell viability. From this practice survival assay, I started to see a decrease in cell viability, possibly indicating that this mutant could not survive in freshwater.

Throughout the funding period, I presented my progress at our lab's weekly meetings where I was able to receive constructive feedback. I was also able to form partnerships with the Gregory Lab, as I participated in and presented at our weekly joint lab meetings with the Ramsey Lab. From these experiences, I was able to collaborate with others as well as getting scientific content from a variety of perspectives.

Lessons Learned

Challenges encountered during this process was troubleshooting the allelic exchange protocol. We had multiple technical difficulties while attempting to make a mutant strain without the *mpl* gene, but I gained additional experience with the protocol, and I learned to have patience. As an undergraduate researcher who did not have prior research experience, I learned valuable technical lessons. A prime example of these technical lessons was learning a molecular technique called colony PCR (verification that our gene was deleted). Having over 40 samples, it was difficult to stay organize with all these samples while also taking the time to undertake the protocol. Taking advice from my mentor, Dr. Kathryn Ramsey, she recommended troubleshooting a protocol with practice samples before I started to use the actual samples. As a novice researcher in the microbiology field, I learned the importance of aseptic technique. In the early stages of my project, I encountered contamination in my samples. With the help of the fellow graduate students in the lab, I learned how to keep my lab environment sterile using a Bunsen burner and proper pipetting techniques. These methods have made me more efficient and has been valuable to the time for this project.

Future Directions

Though this project is not yet finished, the lessons I have learned will have helped me establish valuable skills. I strive to apply these new techniques in the second half of my project which is validating the hypothesis that the gene *mpl* is essential for freshwater survival. I will be continuing to conduct freshwater survival assays, using more techniques that I will become proficient in using. I plan to work efficiently by staying organize and keeping an up-to-date lab notebook of my recorded observations. A long-term outcome I have in plan from the conclusion of my project is to have this work published in a peer review journal, so professionals from a

variety of fields like microbiology, epidemiology and infectious diseases can benefit from these results. I aim to contribute to the collective knowledge of *Fransciella tularensis'* ecology and mechanisms. Finally, I plan to present my project via poster presentation at the Annual Biomedical Research Conference for Minoritized Scientists (ARBCMS) in Philadelphia in November 2024 to share our results.

Acknowledgements

I would like to personally thank the partnerships I have made in this project who have mentored me throughout these past few months. I would like to thank the graduate students, Kira Bernabe, Ben Moore, and Alexandra Farah for their constant feedback. I will also like to thank Dr. Kathryn Ramsey for our frequent meetings about my project but also for professional development.

References

Telford SR 3rd, Goethert HK. Ecology of *Francisella tularensis*. Annu Rev Entomol. 2020 Jan 7;65:351-372. doi: 10.1146/annurev-ento-011019-025134. PMID: 31600457; PMCID: PMC8300880.