

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

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Cell and Molecular Biology

Project Start Date: December 1, 2023

Project End Date: May 3, 2024

Amount of Funding Requested: \$1,000

Introduction

Francisella tularensis is a Gram-negative pathogen and is the causative agent of the infectious disease tularemia. *F. tularensis* is an intracellular pathogen and must replicate inside host cells to cause disease. It is a potential bioweapon and key features that make it a concern for weaponization are its potential to cause lethal disease, that it can be easily aerosolized, and that very few cells can lead to infection (a low infectious dose). While *F. tularensis* can infect mammals like rabbits and humans through insect bites or ingestion of contaminated food/water, it is also found to survive in freshwater environments for an extended period of time (Telford et al., 2020). In this project, we will specifically be using *F. tularensis* subspecies *holarctica* LVS (live vaccine strain) which is attenuated such that it does not cause disease in humans but still retains virulence in animal models. How *F. tularensis* survives in freshwater is still not well understood. We are interested in determining which genes are essential for survival of *F. tularensis* in freshwater. A previous undergraduate in the Ramsey lab carried out a genetic screen that identified the *mpl* gene as a candidate that may be essential for the survival of *F. tularensis* in freshwater. This gene encodes murein peptide ligase, an enzyme important for maintenance of the bacterial cell wall (Hervé et al., 2007). I want to validate her work and determine if the *mpl* gene is necessary for the survival of *F. tularensis* in freshwater.

The overarching goal of this research project is to advance our understanding of *Francisella tularensis* and its survival mechanisms in freshwater environments. We aim to determine if the enzyme encoded by the *mpl* gene is necessary for the survival of *F. tularensis* in freshwater. Using genetic methods to modify the *F. tularensis* genome and freshwater survival assays, we will be able to compare the survival of cells without *mpl* to cells containing *mpl* in freshwater. At the conclusion of this project, I plan to have (1) cloned a plasmid to modify the *F. tularensis* genome, (2) created a strain of *F. tularensis* that lacks the *mpl* gene, and (3) determined the relative survival of different mutants in freshwater, potentially validating the importance of *mpl* to the survival of *F. tularensis* in a key environmental condition.

Approach

The first step in this project is to clone a plasmid to be used in modifying the *F. tularensis* LVS genome. First we amplify (copy) two fragments two pieces of DNA from the genome, from either side of the *mpl* gene. We then purify these fragments and a plasmid backbone. After purification, we use a method called a digest where we cut the DNA into smaller compatible pieces using scissor-like enzymes. We purify the DNA pieces we want to use by running them on a gel,

which separates out different size fragments. Through a process called ligation, we will glue the three DNA pieces (fragment 1, fragment 2 and the backbone) together. We transform the ligations into *E. coli* and grow the bacteria on media that only allows growth of cells with plasmids overnight. We will then perform a miniprep, which is a protocol that allows us to purify the plasmid. Following this, we will perform a diagnostic digest, to confirm if we inserted both of the fragments into the plasmid, and validate that the plasmid sequence is correct by sequencing.

After successfully cloning this plasmid, we will use a method called allelic replacement to delete the *mpl* gene in *F. tularensis* LVS. First, we will transform the plasmid into *F. tularensis* LVS via electroporation (introducing the DNA into the bacteria after disrupting the cell wall by electric pulses). We will grow cells on selective media, so that any cells that grow contain the plasmid DNA. These cells are called primary integrants, because the plasmid should have integrated into the genome. After growing these cells, we will plate them on specific plates that do not allow cells with a plasmid to grow. This will allow only cells that have reverted to the original genomic sequence (wild-type) or deleted the *mpl* gene to live. We will identify the cells lacking *mpl* by amplifying the DNA on either side of the *mpl* gene and confirming the loss by sequencing. These bacteria are now considered a mutant, as they lack the *mpl* gene and we will refer to them as LVS Δmpl .

We will subsequently perform a freshwater survival assay to test the importance of *mpl* for the survival of the bacteria in freshwater. We will test three types of bacteria for their ability to survive in freshwater. These are the standard *F. tularensis* cells (LVS wild-type) which contain *mpl*, LVS Δmpl (mutant with the deletion of *mpl*), and the positive control cells, which lack the gene *FtMcS*. Cells lacking *FtMcS* are a good positive control because it was observed that deleting this gene causes *F. tularensis* to go into hyperosmotic shock, and therefore cannot survive in freshwater (Williamson et al., 2018). These three strains will then be incubated in freshwater and we will take samples over time (up to 2 months) to determine how many cells have survived in freshwater. Expected results for the wild-type LVS would be survival of the bacterium over multiple weeks (as the previous student found). However, for LVS lacking *FTMcS* and *mpl* we expect that we will not identify viable bacteria after a much shorter period of time, possibly as short as one week.

Contribution to the Project

This project started when I learned about a previous undergraduate's research project who proposed the question "what are the genetic requirements for the survival of *F. tularensis* in freshwater?" This undergraduate found that the gene *mpl* was a candidate essential for the survival of the bacterium in freshwater. The gene *mpl* codes for the enzyme that is important for cell wall synthesis. I was excited to see if the gene does play a role in the survival of the bacterium in this important environmental condition. After a conversation with Dr. Kathryn Ramsey, I decided that I wanted to validate these results to see if the gene *mpl* is important for the survival of *F. tularensis*.

Expected Project Outcomes

The findings of this research project hold the potential to benefit the greater scientific community, as the results will help us understand the mechanisms *F. tularensis* uses to survive in the environment. To ensure effective dissemination of results, I plan to present my research at relevant scientific symposiums and conferences such as the Annual Biomedical Research Conference for Minoritized Scientists (ABRCMS). I also plan to publish this research findings in a peer reviewed journal making this information available to the wider scientific audience, particularly professionals in microbiology, epidemiology, and infectious diseases. By sharing the outcomes at these locations, I aim to contribute to the collective knowledge and foster meaningful discussions surrounding *Francisella tularensis* ecology and survival mechanisms.

As a current student majoring in Cell and Molecular Biology on the microbiology track, this research project represents an opportunity for me to build my foundation in molecular biology techniques and immerse myself in the realm of research. Being relatively new to the research landscape, this project serves as a crucial stepping stone to refine my laboratory skills and gain hands-on experience with advanced molecular biology techniques. Beyond technical proficiency, the collaborative nature of this research allows me to engage with my graduate mentor, offering a mentorship opportunity to enhance my understanding of experimental design, data analysis, and scientific communication. This immersive experience aligns seamlessly with my academic goals, providing a platform to cultivate not only technical expertise, but also the critical thinking and problem-solving skills essential for success in a Ph.D. program. **As I aspire to pursue a Ph.D. in biomedical research, this project not only aids in building skill set but also contributes significantly to shaping my identity as a budding researcher, laying the groundwork for impactful contributions to the biomedical research community.**

References

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October 20, 2023

Undergraduate Research Initiative Committee
University of Rhode Island
Kingston, RI 02881

Dear Committee Members,

I am extremely pleased to offer my support to Johanyx Rodriguez for her undergraduate research project, "Assessing the contribution of a cell wall enzyme to survival of *F. tularensis* in freshwater." My laboratory studies the molecular mechanisms that lead to pathogenicity of the human intracellular pathogen *Francisella tularensis*. While this organism is highly pathogenic to humans, my laboratory takes advantage of the model organism *F. tularensis* subsp. *holarctica* LVS (Live Vaccine Strain), which does not infect or cause disease in humans and we do not work with any of the highly pathogenic strains.

Johanyx is an academically excellent upper-level Cell and Molecular Biology student and is a new MARC U*STAR trainee. The goals of the MARC U*STAR program include training students to become critical thinkers, capable researchers, and future scientists, as well as develop a culture of excellence and community in undergraduate research at URI. Acceptance into the MARC U*STAR program is competitive and trainees are well-prepared to begin research. Over the summer, Johanyx participated in "rotations," or trials periods, in multiple research laboratories to identify a scientific group to join for her undergraduate research. The Ramsey laboratory is very pleased to welcome Johanyx to our group and is committed to helping her achieve her research and training goals.

When discussing ongoing research in the Ramsey laboratory, Johanyx expressed interest in continuing work initiated by a former undergraduate student. In particular, a previous student was investigating how *F. tularensis* survives outside of hosts, in only freshwater, for long periods of time. This ability of an intracellular pathogen to persist in the environment is unusual and poorly-understood. This former student undertook a genetic screen and identified a candidate gene which may be important for this long-term environmental survival. Johanyx's interest and goal is to determine if we can validate the hypothesis that this particular gene is important for environmental survival. It is exciting to me that she is interested in this topic because her work would provide insight into understanding how bacteria, and particularly pathogens, persist in the environment and maintain infectious reservoirs.

I think Johanyx's choice in projects will continue to provide new and exciting areas of research for her. If we do validate that this gene is important for environmental persistence, it would be a natural extension for her to study the role of this gene product in bacterial physiology. Alternatively, if her hypothesis is incorrect, it provides an opportunity to revisit the parameters of the genetic screen and improve our methods to find better candidate genes. Together, I enthusiastically support the continued development and undertaking of her independent research project.

Together with my graduate students, I will supervise all the proposed work in this project to ensure safety, rigor, and reproducibility. The success of this project will be based on Johanyx's ability to (i) learn and implement research protocols, (ii) critically analyze the resulting data, (iii)

make connections between the resulting data and the current scientific literature, and (iv) effectively communicate research findings, both within and outside our research group.

Johanyx is an outstanding student with great enthusiasm for laboratory research. Her undergraduate research experience in my laboratory, including the exciting work proposed here, will prepare her to achieve her future goal of entering graduate school in biomedical research. She is extremely well-prepared to carry out the proposed experiments and I support her application for an undergraduate grant for original student research without reservation and with enthusiasm.

Sincerely,

A handwritten signature in dark ink, appearing to read 'KR', with a long horizontal flourish extending to the right.

Kathryn M. Ramsey, PhD
University of Rhode Island
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