

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

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Francisella tularensis is a Gram-negative pathogen, the causative agent of the infectious disease tularemia, and is considered a potential bioweapon. Contaminated water sources that contain *F. tularensis* can be a significant contributor to disease spread, and *F. tularensis* survives in freshwater environments for an extended period of time. However, how *F. tularensis* survives in freshwater is still not well understood. Based on preliminary work, this study aims to determine if the enzyme encoded by the *mpl* gene (murein peptide ligase, an enzyme important for maintenance of the bacterial cell wall) is important for *F. tularensis* survival in freshwater. We would like to test cells that do not create the *mpl* protein to see if they can survive in freshwater. One strategy is to clone a plasmid that would allow us to modify the *F. tularensis* LVS (Live Vaccine Strain) genome to delete, or completely remove, the *mpl* gene. The second strategy is to clone another plasmid to interrupt the *mpl* gene, to preventing production of the functional protein. We have had difficulty deleting the *mpl* gene and are working on creating cells with an interrupted *mpl* gene. Following the production of a mutant, we will conduct a freshwater survival assay to assess the cell viability of *F. tularensis* with the deleted or interrupted *mpl* gene. Our findings are expected to help us understand the mechanisms *F. tularensis* uses to survive in the environment, which may also help us understand how other bacteria survive environmental conditions.