

Research Outline

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Project Title: Determining the genetic requirements for *Francisella tularensis* survival in freshwater.

Description: We want to determine the gene(s) that allow for survival of *F. tularensis* in freshwater. The first aim is to find and validate which temperature condition allows for the long-term survival of *F. tularensis* in sterile freshwater. I investigated the optimal temperature condition in the Ramsey laboratory last semester and, in one experiment, found 4°C to be the optimal condition. Currently, I am validating this finding by replicating my results. I am also creating a vector that will facilitate use of transposon insertion sequencing (Tn-Seq), and I will use it to create a mutant transposon mutant library. We will use this mutant library to investigate which gene(s) are important for the survival of *F. tularensis* LVS at 4°C in freshwater.

Background: *Francisella tularensis* is a pathogenic bacterium and is the causative agent of the disease tularemia. In freshwater aquatic environments *F. tularensis* can survive for long periods of time and is able to transmit and cause disease in animals and humans. Other labs have been able to determine that temperatures near but above freezing allow for longer survival of *F. tularensis* in freshwater. Additionally, this past semester, I found that the attenuated strain of *F. tularensis*, the live vaccine strain (LVS), can survive at 4°C in freshwater for 35 days.

Gap: There is a gap with understanding the genes involved with the long-term survival of *F. tularensis* in freshwater environments. My project aims to determine the gene(s) that are essential for this survival.

Hypothesis: We hypothesize that there are specific gene(s) that are involved in the survival of *F. tularensis* LVS at 4°C in freshwater.

Aims: We will test the hypothesis with the following aims:

1. Determining optimal temperature condition for survival of *F. tularensis* LVS in freshwater
2. Creation of a new vector for Tn-Seq, pKR141
3. Create *F. tularensis* transposon mutant library
4. Determination of gene(s) involved using Tn-Seq protocol (possibly in the fall)

Experiment	Justification	Figure/Data	Expected Results
Aim 1: Optimal Temperature Determination			
Assess bacteria viability at three different temperatures from day 0-62	Determination of which temperature condition in sterile freshwater can <i>F. tularensis</i> survive the longest.	Graphs of viable colony-forming units (CFU) of bacteria, percent of initial inoculum over time, comparison of the three viability CFU experiments, and comparison graphs	4°C will be the optimal for long term survival of <i>F. tularensis</i> in sterile freshwater.
Aim 2: Cloning Plasmid			
Cloning plasmid pKR141	To use technology that is more efficient and cost effective, a new Tn-Seq vector needs to be created	Gels, sequencing results, including plasmid map	New plasmid to use for Tn-Seq.
Aim 3: Mutant Library			
Electroporate pKR141 into <i>F. tularensis</i>	Creation of transposon mutants to be used in following experiment	Images of mutant bacteria colonies	Many colonies that grow on selective media
Aim 4: Tn-Seq			
Grow mutant library for 1 and 2 weeks at two conditions at 4°C; compare with mid-log growth in liquid media	To put the mutants created under the conditions of interest	Graphs of colony forming units survival after 7 and 14 days, purified genomic DNA quality statistics	Purified genomic DNA from viable bacteria.
Tn-Seq Analysis	To determine the gene(s) that are essential for survival in freshwater.	Transposon Insertion Profiles	Determination of the essential and non-essential genes under both conditions. Then determining the essential genes that are specific to the freshwater condition. A list of gene(s) that allow for survival in freshwater at 4°C is the outcome.