Investigating the control of ribosomal protein expression in *Francisella tularensis*

Maria Jose Santiago[1], Hannah Trautmann[2], Jamie Wandzilak[2], Kathryn M. Ramsey[2]

[1]Department of Chemistry and Biochemistry, Florida International University

[2]Departments of Cell and Molecular Biology and Biomedical and Pharmaceutical Sciences, University of Rhode Island

Abstract

Francisella tularensis **is the Gram-negative intracellular bacterial pathogen that causes the disease tularemia and is considered a potential bioweapon. The *F. tularensis* gene encoding the small ribosomal protein bS21-1, *rpsU1*, has been identified as critical for virulence, although it is not known why. Furthermore, the conditions that induce *rpsU1* expression are unknown. In this study, our goal is to investigate how expression of the *rpsu1* gene is controlled in *F. tularensis*. To monitor expression of *rpsU1*, we cloned and utilized an allelic exchange vector to synthesize a reporter strain incorporating the *lacZ* gene downstream of *rpsU1* as a transcriptional fusion. The *rpsU1-lacZ* reporter strain allows us to use production of the protein encoded by *lacZ*, beta-galactosidase, as a proxy for *rpsU1* transcription. Using our *rpsU1-lacZ* reporter strain, we can monitor the transcription of the *rpsU1* gene by measuring the amount of beta-galactosidase in cells grown under different conditions to identify conditions that increase or decrease *rpsU1* transcription. Specifically, we are investigating how temperature and genetic background influence transcription of *rpsU1*. These studies will help us understand the conditions under which the *F. tularensis* virulence factor encoded by *rpsU1* is expressed.**

Introduction

The Gram-negative intracellular pathogen *Francisella tularensis* causes the disease tularemia (Pechous *et al*., 2009). An easily aerosolized and potentially lethal pathogen, *F. tularensis* has been identified as a possible bioweapon (Oyston *et al*., 2015). The *F. tularensis* gene encoding the small ribosomal protein bS21-1, *rpsU1,* has been identified as critical for virulence (Su *et al*., 2007). Why *rpsU1* is important for virulence and how it is regulated are unknown. In this project, we utilized the *F. tularensis* live vaccine strain (LVS), which is attenuated for virulence in humans, to assess the expression of *rspU1* in cells grown in different conditions and determine if those conditions influence transcription of *rpsU1.* This project is divided into two stages: (1) creation of a reporter strain that contains a *rpsU1-lacZ* transcriptional fusion using allelic exchange; and (2) assessing expression of *rpsU1* by performing β-galactosidase assays to determine how much *lacZ* is produced by the *rpsU1-lacZ* reporter strain when cells are grown in different temperatures and genetic backgrounds.

Material and Methods

Stage 1: Creation of the *rpsU1-lacZ* reporter strain
Design the plasmid
PCR Amplification of genes
Restriction enzyme digest
Ligation
Transformation (*E. coli)*
Miniprep
Confirmation by sequencing
Electroporation (*F. tularensis*)
Sucrose selection
Cross out plasmid
Confirmation by colony PCR

Stage 2: Assessing *rpsU1* production in different conditions
Growth of the *rpsU1-lacZ* reporter strain in different conditions
Transposon mutagenesis of *rpsU1-lacZ* reporter strain
β-galactosidase assay to assess how much *lacZ* is produced in *rpsU1-lacZ* cells grown in each condition

Results

Creating the *F. tularensis rpsU1-lacZ* reporter strain.

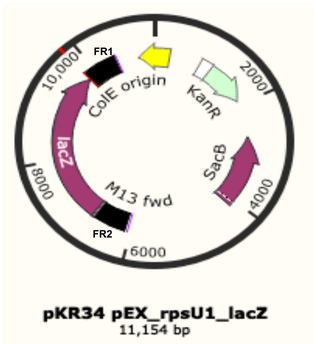

Figure 1. Diagram of the plasmid pKR34, cloned in order to generate the *F. tularensis rpsU1-lacZ* reporter strain

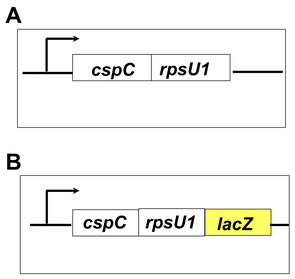

Figure 2. Representation of *F. tularensis* wild type (A) and *rpsU1-lacZ* reporter strain (B)

Expression of *rpsU1* increases at lower temperatures

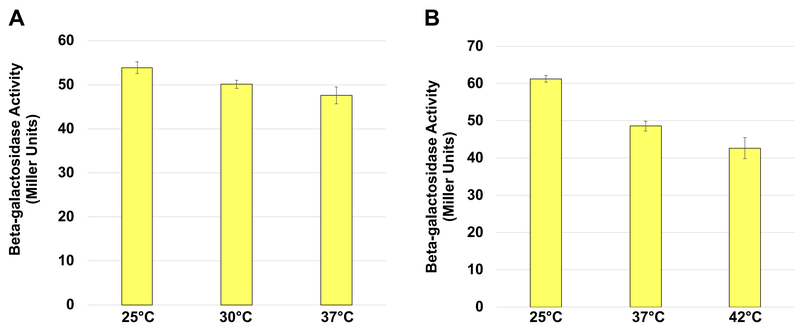

Figure 3. Quantification of *lacZ* expression in the *rpsU1-lacZ* reporter strain grown in indicated temperatures. Cells were grown to mid-log at 37°C, cultures were split and incubated for an additional hour at the indicated temperature. A. First replicate. B. Second replicate.

Identifying genes that influence *rpsU1* transcription using transposon mutagenesis

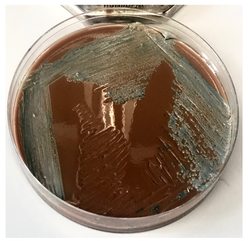

Figure 4. Representative image of the *F. tularensis rpsU1-lacZ* reporter strain struck to single colony on plates containing the colorimetric substrate X-gal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside).

Total number of colonies	Dark Blue	White
1186	159	86
Percent	13.4%	7.3%

Table 1. Summary table of colony phenotypes after transposon mutagenesis. Dark blue colonies are expected to have transposon insertions in genes that negatively regulate the transcription of *rpsu1*, while white colonies are expected to have insertions in *lacZ* or genes that positively regulate the *rpsU1*.

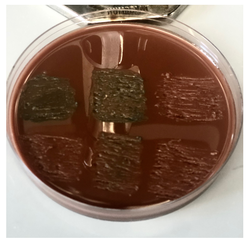

Figure 5. Patches from dark blue single colonies of *rpsU1-lacZ* reporter strain after transposon mutagenesis.

Conclusions

- **Using *E. coli*, we successfully cloned a plasmid to generate the *rpsU1-lacZ* transcriptional fusion.**
- **We created the *F. tularensis rpsU1-lacZ* reporter strain by allelic exchange and used colony PCR to confirm the modification.**
- **We hypothesized that temperature would affect *rpsU1* expression because *rpsU1* is present in an operon downstream of *cspC*, which encodes a cold-shock protein.**
- **Our results from the β-galactosidase assay reveal that temperature affects transcription of *rpsU1*, and specifically that *rpsU1* expression is increased at lower temperatures.**
- **We hypothesized that there may be *F. tularensis* genes which control *rpsU1* transcription. Using a transposon mutagenesis strategy, we were able to inactivate genes that repress expression of *rpsU1*, leading to darker blue colonies in the *rpsU1-lacZ* reporter strain. In future studies, we will identify the genes inactivated by the transposon using arbitrary PCR**

References:

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Acknowledgments

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