THE UNIVERSITY OF RHODE ISLAND

Kathryn Ramsey <kramsey@uri.edu>

# JB00268-22 Decision Letter

**jbjournal@msubmit.net** <jbjournal@msubmit.net> Wed, Aug 3, 2022 at 2:00 PM
Reply-To: pchampio@nd.edu, jbasmusa@gmail.com
To: kramsey@uri.edu

Dr. Kathryn M Ramsey
University of Rhode Island
Kingston

Re: JB00268-22 (A ribosomal protein homolog regulates gene expression and virulence in a bacterial pathogen)

Dear Dr. Ramsey:

Thank you for your submission to JBac. Your manuscript was reviewed by two experts in your field. Both reviewers were very positive about your manuscript, but recommended minor revisions prior to acceptance. I agree with their comments. Please pay special attention to the suggestions regarding the deposition of your raw RNA-seq data to an appropriate repository, as well as the revision of Table 3 to include the entire transcriptome. Please also pay attention to the editorial changes requested by both reviewers. Below you will find the comments of the reviewers.

**Preparing Revision Guidelines**
To submit your modified manuscript, log onto the eJP submission site at https://jb.msubmit.net/cgi-bin/main.plex. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

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Thank you for submitting your paper to JB.

Sincerely,
Patricia Champion
Editor, Journal of Bacteriology

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Reviewer comments:

Reviewer #1 (Comments for the Author):

The manuscript by Trautman and Ramsey describes an interesting case of ribosomal protein bS21 redundancy in Francisella tularensis, which contains 3 homologs of this protein, each incorporated in ribosomes. They show that a loss of the major homolog bS21-2 (found in the equivalent operon as in E. coli), shows defects in the levels of 185 proteins including most of those from the Francisella pathogenicity island (FPI) that govern the production of several type 6 secretion system (T6SS) homologs and the ability to grow in macrophages. The experiments are generally well-performed and the paper is well-written and easy to read. Most of the obvious considerations such as changes in global growth rate that could provide a simplistic explanation for the macrophage growth defect are addressed in complementation studies with the different variants.
My only major criticism is the use of the word 'control' or 'regulation' throughout the manuscript. There is no evidence yet that this phenomenon is regulatory, ie that the levels of bS21-2 can vary throughout the life cycle of Francisella, or in response to changes in environmental conditions, to regulate the expression of FPI mRNAs. The role of bS21-2 could be constitutive and simply reflect different levels of sensitivity of certain mRNAs to bS21 levels that will remain the same regardless of the condition. I am OK with the terms 'determines', 'governs' or even the more nuanced term 'modulate', but without further experiments to show bS21-2 levels vary, the terms 'regulation' and 'control' should be avoided, since these have a specific connotation

Reviewer #2 (Comments for the Author):

Review of Trautmann and Ramsey 2022.

In their manuscript entitled "A ribosomal protein homolog regulates gene expression and virulence in a bacterial pathogen," Trautmann and Ramsey describe their finding that deletion of genes encoding the ribosomal protein bS21 in Francisella tularensis leads to differences in abundance of a number of proteins including several components of the virulence-associated type VI secretion system. There are three bS21 homologs in F. tularensis. Trautmann and Ramsey report that while all three of them appear to be present in ribosomes, bS21-2 is the most abundant. Consistent with this, deletion of bS21-2 had a major impact on the proteome while deletion of the other two did not. Type VI secretion system proteins were particularly affected. These effects could be complemented with ectopic expression of bS21-2 or bS21-3, indicating that there is some level of functional redundancy among the bS21 homologs. However, only bS21-2 could complement an intramacrophage survival defect, indicating that the bS21 homologs have unique functions as well. Overall, the manuscript is well-written, the data are well-presented, and the work itself provides a valuable contribution to the fields of F. turarensis biology and bacterial ribosome/translation biology.

Specific comments:

Raw RNAseq data should be submitted to GEO or another appropriate repository, and the accession number should be stated in the manuscript.

Table S3 should show the data for the entire transcriptome, not just the genes that met the threshold for significance set by the authors. I strongly believe that all 'omics datasets should be published in full, not just subsets of genes selected by the authors.

The authors chose an RNAseq fold-change cutoff to classify genes as having transcript abundance changes or not. Using their cutoff, most genes with altered protein levels did not have corresponding changes in transcript abundance. However, there appears to be a trend towards corresponding changes in transcript abundance for many of these proteins (Figure 2). While this does not affect the conclusions regarding the type VI secretion system genes, I think it should be mentioned as an important caveat to the broader interpretation of bS21 affecting many genes at the level of translation. Many of the genes shown as yellow dots in Figure 2 appear to have small changes in transcript abundance that could explain the differences in protein abundance. It is also possible that many genes have modestly reduced transcript abundance as a consequence of reduced translation leading to faster mRNA degradation. Testing that would require measuring mRNA half-lives, which is beyond the scope of the current study, but the idea should be mentioned.

Given that bS21 is predicted to exert its function during translation initiation, it would be nice to see a little bioinformatic analysis of the ribosome binding site sequences of genes that are affected by bS21-2 deletion at the translational level vs genes that are not affected. It would be interesting to see if the affected genes differ with respect to Shine-Dalgarno sequence or with respect to the spacing between the SD sequence and the start codon. However, I understand this may be beyond the scope of the current study and I don't think it's necessary for publication.

There is frequent switching between the past and present tense. The manuscript should be edited to use the past tense for all descriptions of data.