THE UNIVERSITY OF ALABAMA IN HUNTSVILLE | COLLEGE OF SCIENCE

Research and Creative Experience for Undergraduates (RCEU) Program 2023

Describing the effects of antibiotic-resistance ribosomes on gene expression in bacteria

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Introduction

Interactions between the compartments of the ribosomal exit tunnel and newly synthesized proteins can impact whether expression of a particular gene will continue. Mutations in this region are associated with antibiotic resistance. Previous research indicates that the K90D mutation in UL22 protein decreases the expression of the lysine-dependent acid-resistance gene, CadB in E.coli. This project was the first attempt to replicate those results as well as investigate how CadB is affected by the +A751ins mutation in 23s rRNA.

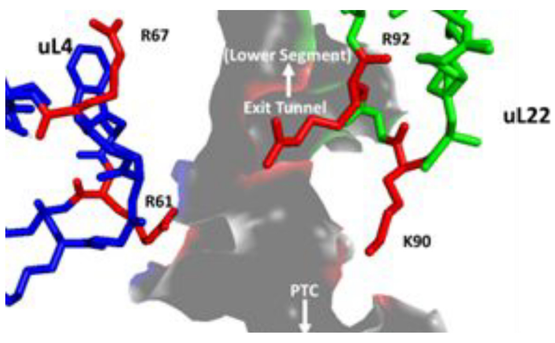


Figure 1. Model of protein compartments within the bacterial ribosomal exit tunnel, including the location of the amino acid residue affected by the K90D mutation.

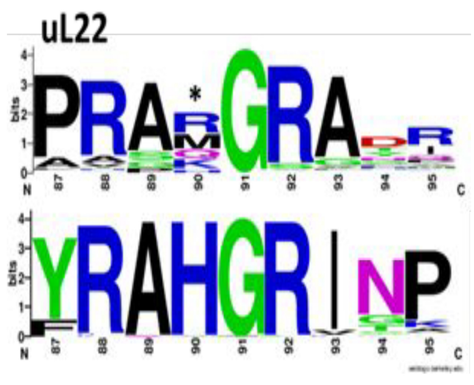


Figure 2. Represents the regularity at which certain amino acids appear in specific locations along the primary structure of the UL22 protein.

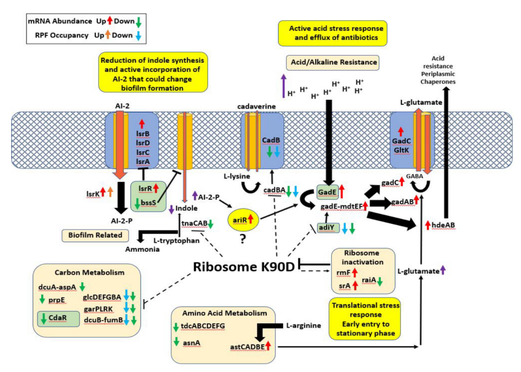


Figure 3. Map of the regulation of the expression of the Cad operon.

Experimental Methods and Results

Two different cell strains were used to test each mutation's effect; K90D was tested in SVS1144 and +A751ins was tested in MG1655 Δ7. CadB expression was determined through the detection of a green fluorescent protein (GFP) expressed by a transcriptional fusion and a protein fusion plasmid. GFP levels were observed through confocal microscopy and western blotting under neutral and acidic conditions. The construction of growth curves aided in determining whether the mutations affected bacterial growth and what the ideal time to let cells grow before observing gene expression was.

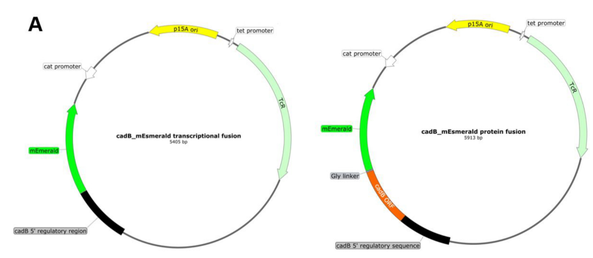
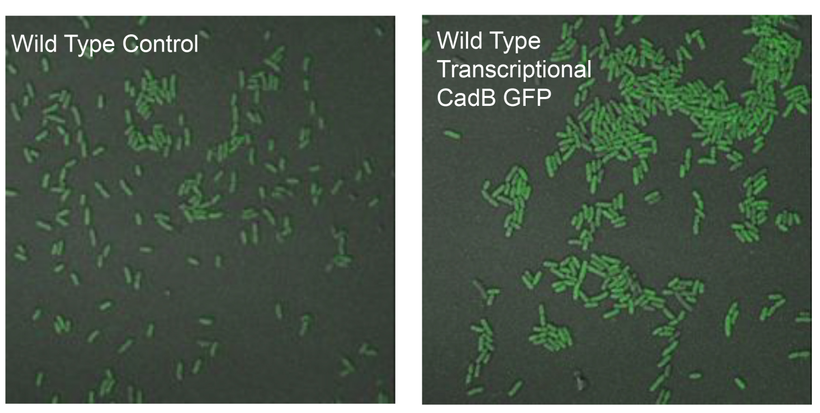
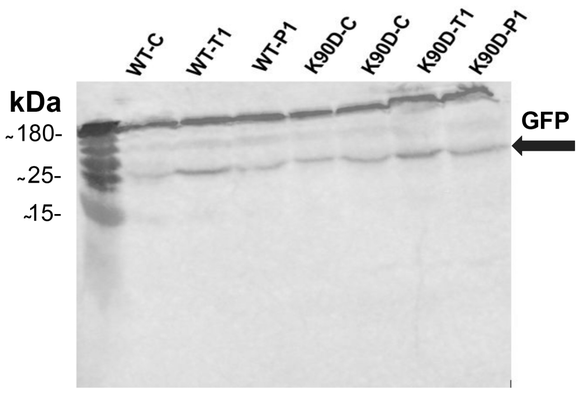


Figure 4. Structures of plasmids used to track expression of transcriptional activity (left) and translational activity (right) of cadB.





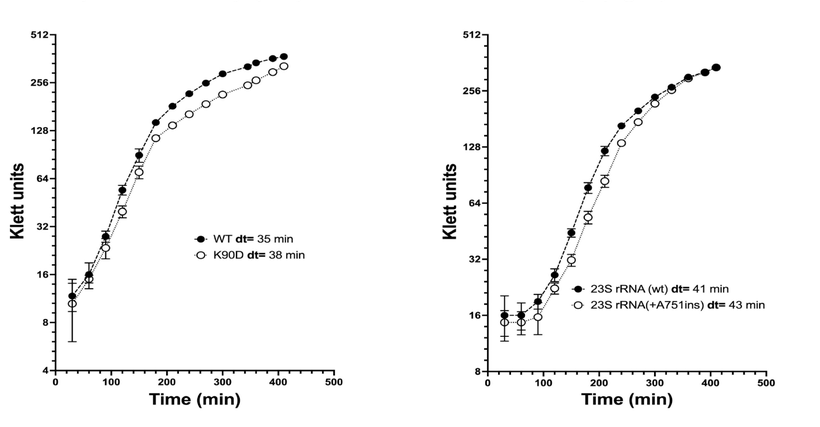


Figure 5. Curves of the growth of Wild Type SVS1144 compared to K90D SVS1144 (Left) and Wild Type MG1655 Δ7 (Left) compared to +A751ins MG1655 Δ7 (Right).

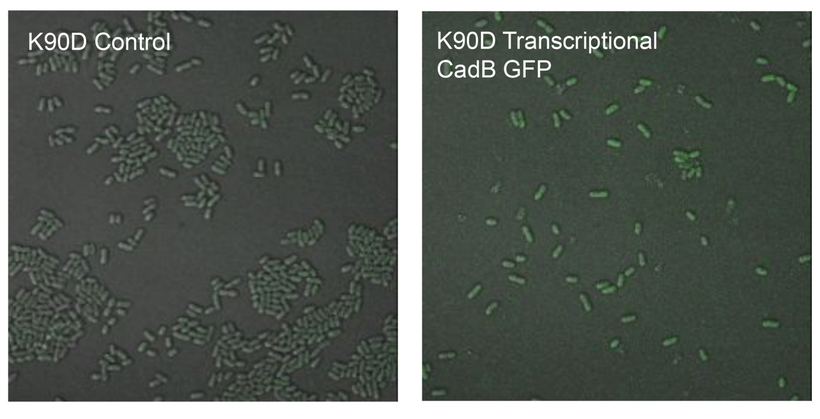


Figure 6. Micrographs collected from Zeiss confocal microscope depicting GFP expression in SVS1144 Wild Type ps10 (top left), Wild Type Transcriptional fusion (top right), K90D ps10 (bottom left), and K90D Transcriptional fusion (bottom right).

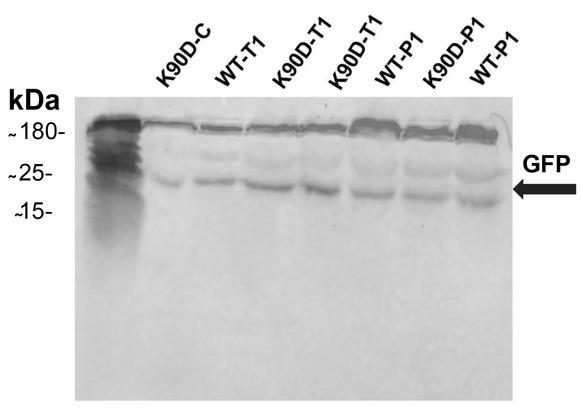


Figure 7. Results derived from western blot used to detect the presence of GFP among Wild Type and K90D cells of SVS1144 E.coli containing no plasmid, a transcriptional fusion plasmid, and a protein fusion plasmid.

Acknowledgements

All RCEU projects were sponsored in part by the Alabama Space Grant Consortium, the UAH Office of the president, Office of the Vice President for Research and Economic Development, the Deans of the College of Science, the College of Engineering, the College of Arts, Humanities, and Social Sciences, the College of Education, and the College of Nursing. I would also like to acknowledge Dr. Kraemer for his help with the confocal microscopy, as well as Dr. Lawan, Nabin Ghimire, and Kaitlin Bryce for their help with the production of the western blots.

References

1. **Worthan, S. B., Franklin, E. A., Pham, C., Yap, M. N. F., & Cruz-Vera, L. R. (2022). The Identity of the Constriction Region of the Ribosomal Exit Tunnel Is Important to Maintain Gene Expression in Escherichia coli. Microbiology Spectrum, 10(2), e02261-21.**
2. **Neely, M. N., & Olson, E. R. (1996). Kinetics of expression of the Escherichia coli cad operon as a function of pH and lysine. *Journal of bacteriology*, *178*(18), 5522-5528.**