5-2022

Investigating the Role of bS21 in Vancomycin-Resistant Staphylococcus aureus

Daniel Floyd
daniel_floyd@uri.edu

DAN FLOYD (Cell & Molecular Biology: Biochemistry)

Investigating the role of bS21 in vancomycin-resistant *Staphylococcus aureus*

Sponsor: Kathryn Ramsey (Cell & Molecular Biology, Biomedical & Pharmaceutical Sciences)

Staphylococcus aureus is a Gram-positive pathogenic bacterium commonly known for being the causative organism of staph infections in clinical settings. In previous literature, it has been observed that mutations in certain genes in *S. aureus* can result in resistance to commonly used antibiotics. Specifically, mutations in the gene rpsU, encoding the ribosomal protein bS21, have been present in strains of *S. aureus* resistant to the antibiotic vancomycin. The aim of my project is to validate these findings by creating a strain of *S. aureus* without bS21 and performing an antibiotic assay to determine the level of resistance to vancomycin in this strain. To do this, I have been working to create a plasmid that can be introduced into wild-type *S. aureus* and knock out the gene *rpsU* via homologous recombination. To create the desired plasmid, I am modifying the pIMAY-Z plasmid which contains the gene for chloramphenicol resistance; I began by amplifying two desired sequences (also referred to as flanking regions) via PCR that will be inserted into the plasmid. I then performed a restriction enzyme digest to cut the plasmid at the insertion sites, along with cutting the amplified sequences at their ends so that they can be added to the plasmid. I continued by performing a ligation to join the flanking regions to the ends of the pIMAY-Z backbone and effectively “glue” the entire plasmid together. I used this ligation to transform chemically competent IMO8B *E. coli* cells on Luria-Bertani agar plates containing 25 ug/ml chloramphenicol, which amplified the desired plasmid for use in further steps. Although I have not confirmed whether this plasmid has been successfully produced through the digests, ligations, and transformations I have performed so far, the positive and negative controls have been successful, and I am now just waiting on official confirmation through sequencing.