

2019 Academic Year Undergraduate Research Grant Final Report

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Throughout this project, I attempted to characterize the fundamental processes underlying antibiotic resistance in *Pseudomonas stutzeri* and *P. fulva* based on the previously characterized process in *P. aeruginosa*; mutations in the efflux pump, encoded for by the *mexA*, *mexB*, and *oprM* genes, are correlated with reduced resistance to certain antibiotics, such as triclosan, in *P. aeruginosa*. In order to assess if this mechanism is conserved across strains, I performed transposon mutagenesis which involves transforming a strain of *Pseudomonas* with a plasmid from *E. coli* that contained a transposon and a gentamycin resistance selection marker. After taking up the plasmid, the transposon was randomly inserted into one out of sixty one insertion sites within the *Pseudomonas* genome, effectively knocking out the function of that specific gene. In this project, the homolog of the *mexAB oprM* operon was the gene of interest as it is known to encode for a multidrug efflux pump in *P. aeruginosa*. If the transposon inserts into a gene responsible for antibiotic resistance, this phenotype is knocked out and the mutant colony is susceptible to triclosan (TCS). I chose TCS as the antibiotic to test for resistance levels and changes in susceptibility as I had previously determined that these two wild type strains were resistant to TCS. Therefore, I hypothesized that if the transposon is inserted in to the *mexAB oprM* homolog, then the mutant colony will be less resistant to TCS. Finally, colonies appearing to have less resistance were sequenced in order to determine the exact insertion site and location of the gene that, when present, contributes to antibiotic resistance.

After conducting mutagenesis and generating 4,000 *P. fulva* and *P. stutzeri* mutant colonies, I have learned about the molecular mechanisms behind resistance in these strains. Seven of the 4,000 *P. fulva* mutants exhibited a decrease in the minimal inhibitory concentration of triclosan from 128.0 mg/L to 8.0 mg/L, indicating that these mutants were significantly less resistant to TCS after mutagenesis. After sequencing the seven *P. fulva* mutant colonies, it was determined that the transposon had inserted itself into, and disrupted the function of, the homologous *mexAB oprM* operon. This discovery indicates that the mechanism of resistance is highly conserved between *P. aeruginosa* and *P. fulva* and the homologous operon performs a very similar function in both strains. Interestingly, this was not the case for *P. stutzeri*. While the seven mutant colonies were unable to grow on plates containing TSA + TCS with a TCS concentration of 256 mg/L after mutagenesis, when I conducted further testing to determine the minimal inhibitory concentration, I discovered that the minimal inhibitory concentration has actually increased from 1.0 mg/L to 4.0 mg/L instead of decreasing indicating that the mutant colony was actually more resistant to TCS than the wildtype colony. These results are inconsistent with my hypothesis that the mutant colonies would be more susceptible to antibiotics; however, I have yet to determine the location of the insert in these colonies. I am currently in the process of further investigation as to why resistance to TCS appeared to increase in all seven mutant *P. stutzeri* colonies and have sent the colonies out for sequencing to determine the location of the transposon insertion. If the transposon did not insert itself into the homologous *mexAB oprM* operon, then this result would indicate that the resistance mechanism

is likely not conserved between *P. aeruginosa* and *P. stutzeri* but it is conserved between *P. aeruginosa* and *P. fulva*.

Next, I am going to attempt to characterize the mechanisms of resistance in *P. fluorescens*. I would like to determine if the *mexAB oprM* operon is conserved across these three strains as I already have discovered it contributes to resistance in *P. fulva* and know from previous research its role in *P. aeruginosa*. By explaining how this system works in *P. fluorescens* and *P. stutzeri*, I will further our understanding of antibiotic resistance in general. I would like to find out how differences in the amino acid sequences of the efflux pump encoded for by the *mexAB oprM* operon. I would also like to evaluate the genetic differences between the homologous *mexAB oprM* operon between *P. fulva*, *P. stutzeri*, *P. fluorescens*, and *P. aeruginosa* as the difference in results between *P. fulva* and *P. stutzeri* may indicate significant differences in the genes as well as the proteins encoded for by this operon resulting in different resistance mechanisms. It is necessary to examine these differences in order to determine the underlying causes of resistance to, hopefully, develop more advanced ways to fight infections. Finally, I plan to take this project further by performing gene deletion and *sacB* mediated recombination in *P. stutzeri* over the summer. The purpose of this project is to further expand on my current project by determining whether the *mexAB oprM* operon homolog is constitutively expressed in *P. stutzeri* as this homolog does not contain a proximal regulator such as the one in *P. aeruginosa*. The lack of such a regulator could explain the difference in results of the transposon mutagenesis experiment. This project will also allow me to determine if the MexAB OprM efflux pump in *P. stutzeri* effluxes the same antimicrobial products as *P. aeruginosa*. The purpose of this project is to create a more in depth characterization of the efflux pump responsible for antibiotic resistance in multiple strains of *Pseudomonas* as *P. aeruginosa* is an opportunistic pathogen that causes complications in cystic fibrosis patients. Therefore, gaining this new information about the mechanisms that render our extant antibiotics ineffective may allow us to develop novel and more effective treatments.