Characterizing the Role of PA2803 Protein in Antibiotic Resistance in Bacteria

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Pseudomonas aeruginosa is a human pathogen and a leading cause of death in cystic fibrosis (CF) patients. This bacterium is well-known for its antibiotic-resistance, including the “last resort” antibiotic polymyxin-B (PolB). We have observed that P. aeruginosa becomes more resistant to PolB in the presence of elevated Ca levels, like those seen in the lungs of CF patients. We have identified three proteins that are related to said Ca induced PolB resistance. The goal of this project is to purify one of these proteins, PA2803, and to characterize it to better understand its role in the above-mentioned Ca-induced resistance. To achieve this goal, the DNA encoding the PA2803 gene was isolated and amplified through PCR, and was cloned into plasmid, PSKB3; the sequence was verified, and the resulting cloned vector was transformed into Escherichia coli DH5a for maintenance. The cloned vector was also transformed into E. coli BL21 (DE3) for expression of the protein. In order to express the protein, the protein purification process was first optimized via four temperatures, eight incubation times, and six IPTG concentrations, the inducing agent; the optimized process was determined to be cultures grown at 37?, incubated for 16hrs, and induced with a final concentration of 15 uM of IPTG. Using this optimized process, we intend to purify PA2803 and subject it to enzyme assays, in order to describe the protein’s function. Furthermore, we are currently cloning an over-expression vector to over-express PA2803 in P. aeruginosa to better understand the role of the protein via the resulting change in phenotype.

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