Gene Identification in *Serratia marcescens* Polymyxin B sensitive mutants

Ndukwe, Miriam[1], Lindsay, Charmaine[2] and Harris, Randall[3]

[1]Undergraduate Research Assistance, Biology Department, Claflin University, [2]Undergraduate Research Assistance, Biology Department, Claflin University, [3]Associate Professor, Biology Department, Claflin University

mndukwe@claflin.edu

Keywords: Keratitis, Cation Antimicrobial Peptides, Polymyxin B, *Serratia marcescens*

Abstract: *Serratia marcescens* is a gram-negative rod, facultative anaerobic and mobile bacterium. It is within the family Enterobacteriaceae. It could utilize a variety of nutrients and grow under extreme conditions. Serratia causes many diseases such as bacterial keratitis, purulent conjunctivitis, corneal cancers and endophthalmitis. Keratitis is the infection of the cornea in the eye and when left untreated could lead to legal blindness. *Serratia marcescens* possess genes that makes it resistant to the cationic antimicrobial peptides, CAMPs. The experimental purpose was then to find out what kind of genes makes it resistant to these CAMPs. Polymyxin B, a type of CAMPs was used in this experiment. Mutant R7-5A from previous research that was sensitive to Polymyxin B was tested. The DNA of the mutant was digested with enzyme BamH1 after it was isolated and purified. The digested DNA was then self-ligated using T4 DNA ligase. The DNA at ends of the transposon which was inserted into the gene was amplified by PCR. The amplified PCR product was then separated on a 1% agarose gel. The purified PCR product from the gel was ligated into pJET1.2 plasmid. Chemically treated Escherichia coli were then transformed with the ligation mixture on LB agar plates with ampicillin to give a plasmid which contains the transposon. After the transformation process, six colonies were found on the LB agar plates. The plasmid DNA of the colonies was isolated, purified and digested with a Bgl 11 digest. The plasmids were then sent to Eurofins Genomics for sequencing. The transposon was then found to be located within the ArnT gene. Previous research shows that ArnT pathway is needed for Polymyxin B resistance. The ArnT pathway synthesizes aminoarabinose which is added to the lipopolysaccharide in the outer cell membrane. Further research includes identifying the genes that are mutated in one other mutant and to develop assays to examine how the genes are involved in Polymyxin B resistance.