**Serratia marcescens Quorum Sensing Dependent Biofilm Formation**

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**ABSTRACT**

Heavy metals are harmful and they cannot be biodegraded easily. Due to the research, development and production of nuclear weapons at Savannah River Site (SRS), a number of areas at this site are contaminated with heavy metals. This pilot research project supports the environmental remediation efforts at SRS. *S. marcescens* reduces the heavy metal chromium (VI) to the less toxic chromium (III). *S. marcescens* biofilm formation can prevent bacterial movement through contaminated soils. This research is focused on investigating quorum sensing dependent biofilm formation to improve the bioremediation capacity of *S. marcescens*. Quorum sensing enables bacteria to act as one living system by the release of the signaling molecule acyl homoserine lactone (AHL) to coordinate their behavior and execute a phenotypic change such as biofilm formation. Transposon mutagenesis generated ~5000 mutants that were screened for loss of AHL production using the Chromobacterium violaceum biosensor CV026. Five mutants were isolated that had reduced biofilm formation after 6 hours. The mutated genes in these mutants will be identified.

**RESULTS**

Figure 1: Biofilm Formation

Figure 2: Screening of Mutants

Figure 3: Acyl Homoserine Lactone

Figure 4: Biofilm Assay

Figure 5: Biofilm Assay Results

Figure 6: Crystal Violet Absorbance Readings

**METHODS**

The plasmid DNA was isolated from the Escherichia coli DH5α, per containing pTnMod-RKm through the use of a high-speed plasmid mini kit protocol. The plasmid was then transformed into the E. coli BW29247 strain and plated on a LB/diaminopimelic acid/kanamycin plate and incubated at 37°C for 24 hours. Since the plasmid has a kanamycin resistance gene on it, only the E. coli bacteria that took up this plasmid will grow on the plate. A conjugation was performed overnight at 25°C between S. marcescens and the transformed E. coli. The S. marcescens and E. coli mixture, after incubation, now has the potential to have *S. marcescens* mutants and was plated on LB/kanamycin plates. The BW29247 strain of E. coli used in the conjugation does not have the ability to synthesize diaminopimelic acid (DAP) and thus will not be able to grow without it in the medium. The resulting colonies will consist of only *S. marcescens*.

**CONCLUSIONS**

We were successful in generating a mutant library of *S. marcescens* with a reduced ability for quorum sensing and biofilm formation. Five mutants produced lower levels of AHL. This suggests that there was a possibility that one of the genes involved in quorum sensing was mutated. After the biofilm assay, all of the mutants had a smaller average absorbance than the wild type at 6 hours, but all of them had a greater average absorbance than the wild type at 24 hours.

**FUTURE PLANS**

Examine biofilm formation at other time points, e.g., 12 hours and 48 hours. Determine the genes mutated by cloning and sequencing the DNA next to the transposon and comparing the sequence to the NCBI GenBank database.

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**REFERENCES**