Serratia marcescens Quorum Sensing Dependent Biofilm Formation

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ABSTRACT

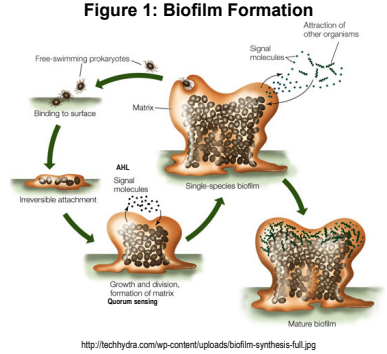
Heavy metals are harmful and they cannot be biodegraded easily. Due to the research, development and production of nuclear weapons at Savannah River Site (SRS), a number of areas at this site are contaminated with heavy metals. This pilot research project supports the environmental remediation efforts 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.

INTRODUCTION

Bioremediation is a process whereby a biologic agent is used to break down or reduce a harmful contaminant present in soil or water in order to reduce the contamination of that substance. With the increasing cases of pollution, bioremediation is becoming more important. Bacteria can reduce pollutants but a problem arises when the bacteria attaches to substances in the soil and form biofilms. While bacterial biofilms can be adsorb contaminants in soils and ground water, the bacteria are prevented from penetrating further into soils or sediments, reducing their bioremediation potential. This research seeks to enhance the potential of *S. marcescens* in bioremediation by examining the the role quorum sensing plays in biofilm formation. Understanding this process will allow us to devise methods to manipulate it to enhance bioremediation. The methods can be applicable in other research areas such as biofouling where bacteria form biofilms inside of pipes and clog them which prevents industrial processes from working efficiently.

RESULTS

Figure 1: Biofilm Formation



http://techhydra.com/wp-content/uploads/biofilm-synthesis-full.jpg

Figure 2: Screening of Mutants

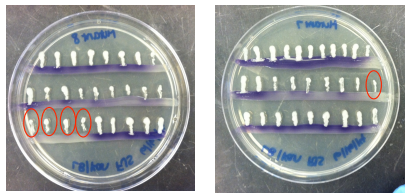


Figure 3: Acyl Homoserine Lactone

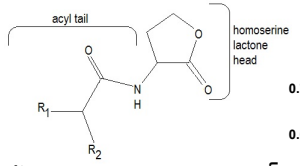


Figure 4: Biofilm Assay

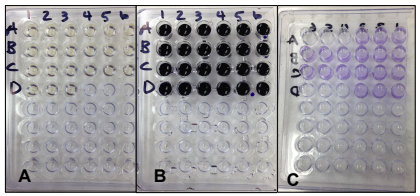


Figure 4A shows the PVC plate with the bacteria in LB media.
Figure 4B shows the wells incubated with crystal violet dye.
Figure 4C shows the biofilm left on the wells. Plate is inverted.

Figure 5: Biofilm Assay Results

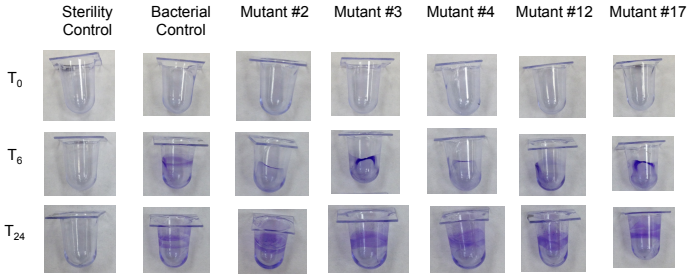
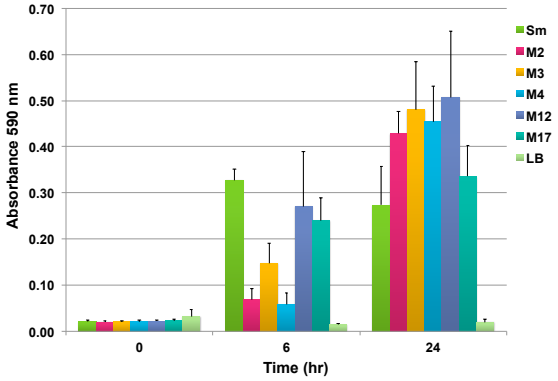


Figure 6: Crystal Violet Absorbance Readings



METHODS

Transformation, Conjugation, & Transposon Mutagenesis

The plasmid DNA was isolated from the *Escherichia coli* DH5α λ pir containing pTnMod-RKm' through the use of a high-speed plasmid mini kit protocol. The plasmid was then transformed into the *E. coli* BW29427 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 BW29427 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*.

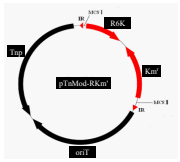


Figure 1: Map of pTnMod-RKm' plasmid. The transposon contains inverted repeats, a replication origin, and the gene for kanamycin resistance. The transposase gene and origin of transfer for conjugation are outside of the transposon on the plasmid. The plasmid replicates in *E. coli* and not in *S. marcescens*. The transposase randomly integrates the transposon at different locations into the *S. marcescens* DNA.

Mutant screening

The biosensor *Chromobacterium violaceum* CV026 detects acyl homoserine lactones with a 4-6 carbon acyl group. When the AHL is present, CV026 produces a purple pigment. The mutants were placed on a plate near the CV026, along with the original *S. marcescens* strain for control as shown in figure 2. If the production of the pigment by the CV026 is noticeably less or absent than the control, then the AHL production in the mutant has been compromised.

Bofilm Assay

A 3 ml culture of the mutants of interest was grown out overnight and then diluted 1:100. A 96 well PVC plate was inoculated with 100 µl of each mutant, as well as the original *S. marcescens*, and a sterility control of just the broth. At 0 hrs, 6 hrs, and 24 hrs, the growth in the wells was washed out and stained with 0.1% crystal violet solution. After being rinsed out again, the wells were incubated with ethanol at room temperature, and then the optical density was measured at 590 nm for each and used for comparison.

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.

REFERENCES

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