Identification of *Serratia marcescens* Genes Needed for Polymyxin B Resistance

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ABSTRACT

Over 30 million people in the United States wear contact lenses. Keratitis is an infection of the cornea associated with the improper care of contact lenses and known to cause legal blindness. *Serratia marcescens* is a gram-negative bacterium known to cause keratitis and is resistant to the antibiotic polymyxin B. Polymyxin B is a cyclic cationic peptide similar to antimicrobial peptides made by the cornea. We hypothesized that *S. marcescens* genes responsible for polymyxin B resistance are also needed for resistance to corneal antimicrobial peptides. Previously, we used transposon mutagenesis to generate five polymyxin B sensitive *S. marcescens* mutants. This project focuses on identifying the gene conferring resistance to polymyxin B in one of the *S. marcescens* mutants. Genomic DNA was isolated from the mutant and digested with the restriction enzyme *Bam*HI. The DNA was purified and the ends of the DNA were self-ligated. The ligation reaction was transformed into *Escherichia coli*. This process converts the transposon and adjacent DNA into a plasmid. The plasmid was isolated from *E. coli* and the DNA next to the transposon was sequenced. The sequence was compared to the GenBank database at the National Center for Biotechnology Information. Sequence analysis indicated that the transposon inserted in between two genes encoding an oxidoreductase and a LysR type regulator. These genes are predicted to be transcribed in opposite directions. Future experiments will be to determine if the transposon interferes with transcription of the gene.

INTRODUCTION

There are over 30 million people in the United States that wear contact lenses. Contact lenses help improve vision without drastically changing a person's appearance. However, these medical devices are often not taken care of properly. Between 40% and 90% of those contact lens wearers do not follow the instructions given to them by their health care providers to properly take care of their contact lenses. Improper care of contact lenses can lead to the formation of a biofilm on the contact lenses. Once that biofilm has formed and the contact lens comes in contact with the eye, keratitis, an infection or inflammation of the cornea, can occur. Keratitis can have the major consequence of loss of vision or legal blindness if left untreated. Each year, bacterial resistance continues to be an issue in the United States and in health care facilities such as hospitals and nursing homes. Because bacteria are becoming more and more resistant, it is becoming increasingly difficult to treat infections and conditions caused by bacteria.

Serratia marcescens is a gram-negative bacteria commonly found almost anywhere in nature. This bacteria is a part of the genus *Serratia*, which has 14 species recognized within it. Out of these 14 species, 8 are associated with human infection. *Serratia marcescens* is the most common species of the genus *Serratia* that is known to be a human pathogen. *Serratia marcescens* would commonly give off a red pigment, which made it a good biological marker for infection. Though this red pigment was present in environmental strains, some non-pigmented isolates were found to be the cause of infection in health care facilities. 15-30% of all cases of keratitis are caused by *Serratia marcescens.*

The corneal epithelium produces antimicrobial peptides which is a natural defense mechanism used to fight off infection. As *Serratia marcescens* continues to evolve and act as a superbug, these antimicrobial peptides are not having the capabilities to fight off the keratitis that is caused. Not only are the antimicrobial peptide defenses being bypassed, but so are the defenses of antibiotics such as polymyxin B. Polymyxin B is an antibiotic which is commonly found in Neosporin. Once a common treatment of infection caused by gram-negative bacteria, the use of polymyxin B slowly dwindled down due to concerns about its toxicity level. With increasing bacterial resistance occurring, polymyxins are making a comeback.

Using transposon mutagenesis, five mutants were identified as being more susceptible to the cationic antimicrobial peptide polymyxin B. Now that susceptible mutants have been indentified, my project is to determine what gene within the genome of *Serratia marcescens* allows it to confer resistance to polymyxin B.

RESULTS

Figure 1. Gel Electrophoresis of Restriction Digest

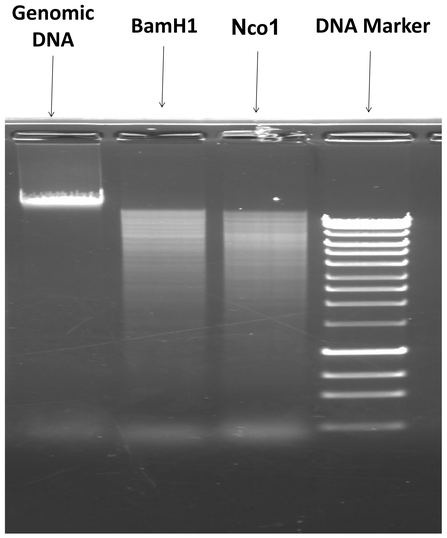


Figure 2. *Escherichia coli* Transformation

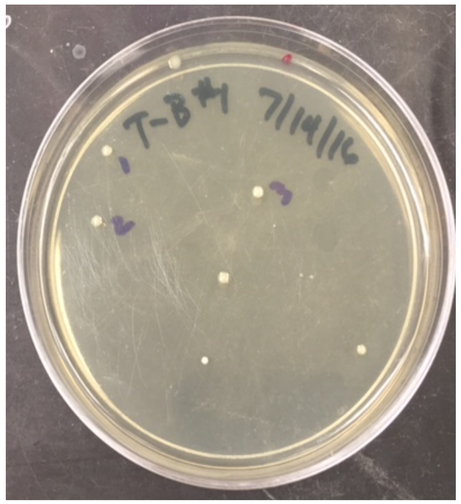


Figure 3. Gel Electrophoresis of Digested Plasmid Clones

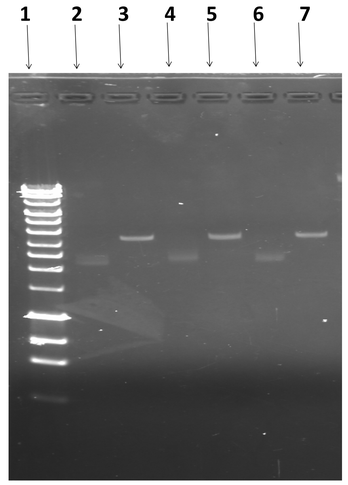
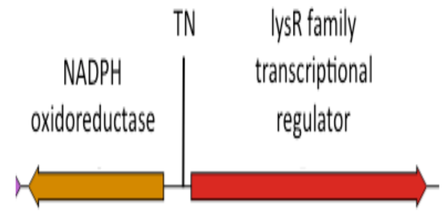


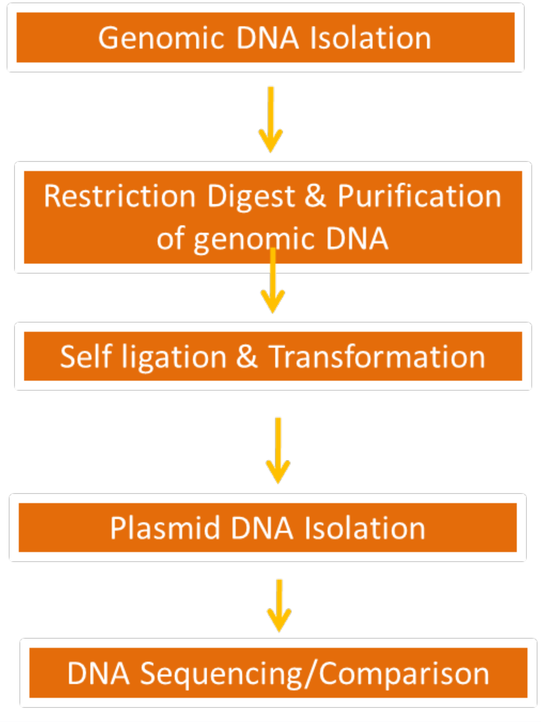
Table 1. Legend of Lane Components in Figure 3

1. DNA Marker
2. Undigested Clone 1
3. BamH1 Digested Clone 1
4. Undigested Clone 2
5. BamH1 Digested Clone 2
6. Undigested Clone 3
7. BamH1 Digested Clone 3

Figure 4. Transposon Placement after Sequencing



MATERIALS AND METHODS



CONCLUSION

Instead of inserting itself in between one gene, the transposon of mutant 1J inserted itself between two genes: NAPH oxidoreductase and lysR family transcriptional regulator.

FUTURE PLAN

I am currently working to determine which gene, if not both, were mutated by the transposon.

This is being done by isolating the RNA of the wild type and mutant to compare expression of the lysR gene or the oxidoreductase gene between the two bacteria.

Reverse Transcription – PCR will be used as the protocol.

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