Determination of the antibiotic resistant capabilities of two putative beta lactamase genes, Bla534 and Bla63, in *Elizabethkingia meningoseptica*

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Abstract

Elizabethkingia meningoseptica is a bacterium prevalent in nature. The *Elizabethkingia* genus has two other discovered species: *E. anopheles*, and *E. miricola*. This genus of bacteria has been known to contribute to meningitis in humans. Little is known about the genome of this genus, and in this experiment start testing two supposed beta- lactamase genes in *E. meningoseptica*, Bla534 and Bla63, to see if they contribute to antibiotic resistance. The two genes are in various stages of getting prepared to test the bacteria for antibiotic resistance. We used PCR, gel electrophoresis, DNA ligation and transformation to insert the two specific *E. meningoseptica* genes into *E. coli*.

Keywords: *Elizabethkingia meningoseptica*, beta lactamase

Introduction

Our group mainly focused on the species of *Elizabethkingia meningoseptica*, because it is the most clinically relevant. *E. meningoseptica* flourishes in wet conditions and hospital settings. This produces the possibility of this bacteria living in the water and saline solutions used to treat patients. *E. meningoseptica* is well known as a major contributing agent of neonatal sepsis and meningitis. The bacterium is also highly resistant to antibiotics, which makes it very difficult to treat. *E. meningoseptica* can spread quickly and has been responsible for disease outbreaks in many hospitals. Unfortunately, nosocomial infections caused by *E. meningoseptica* can result in mortality rates as high as 52% (Teo *et al.* 2014).

The reason *E. meningoseptica* is so resistant to common antibiotics like penicillins, cephamycins, and carbapenems is because certain genes in the bacteria code for a protein that produces an enzyme called beta-lactamase. Beta-lactams make the bacteria resistant to antibiotics by hydrolyzing the antibiotics before they can enter the cell. This causes the deactivation of the antibacterial properties of many drugs used to treat *E. meningoseptica* (Neu 1969). In our research, we are attempting to determine if two of the putative beta-lactamases are responsible for antibiotic resistance. Hopefully this information could aid physicians with treating *E. meningoseptica*.

Methods

We amplified two Bla genes in *E. meningoseptica*, Bla543 and Bla63. We performed polymerase chain reaction on our genes (PCR) by combining various reagents: dH2O, 5xHF, dNTD, template, forward and reverse primer, $MgCl_2$, DMSO, Phusion Taq. These reagents were added to a tube, and run in a thermocycler, or PCR machine, and put through a series of temperature changes. The thermocycler starts at 60° C in order to anneal the primers, rises to 72° C to polymerize the DNA, then rose again to 95° C for a short amount of time in order to slightly denature the DNA allowing for the PCR process to occur.

We assayed the results of our PCR amplification, via gel electrophoresis on our PCR product. This separates DNA fragments and filters them by size. Our PCR products were shown to have approximately 1200 base pairs.

Next we cleaned up our PCR product, removed excess proteins, through a series of phenol chloroform extractions. In these extractions, we centrifuged the DNA with phenol chloroform and then extracted the DNA from the mixture. Following the phenol chloroform extractions, we Nano Dropped our PCR product. The Nano Drop is an analysis performed by a computer and machine which are able to calculate the amount of DNA in our sample. We then repeated these two steps, phenol chloroform

*=Mentor

Figure 1 *- Results from the Gel Electrophoresis of our PCR Product. Each of the three PCR samples align with the 1200 base pair marker on the PCR ladder, confirming that the PCR of Bla63 was successful*

Figure 3a Figure 3b

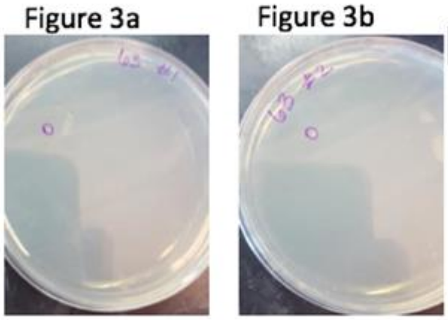

Figure 3a & b *- Results of transformation opfBla63 ligation into* E. coli *cells*

extraction and the Nano Drop. (At this point Bla543 was not taken further and was not ligated due to errors in the lab)

Following the second Nano Drop, we ligated our genes in kanamycin resistant plasmid vectors. We did this by combining dH_2O 11 µL, 10x DNA ligase buffer 2 µL, Vector DNA 2 µL, Insert DNA 4 µL, and DNA ligase 1 µL. We incubated the ligation overnight. Due to an error with the plasmid vectors, we did the ligation for Bla63 a second time.

After adding 2 mL of our ligation to *Escherichia coli* cells, we then heat shocked this combination. We then put this combination on ice for 30 minutes. After the 30 minutes, we put it in a water bath at 42° C for approximately 30 seconds and then placed the mixture on ice for 3 minutes before letting it sit at room temperature.

Following the transformation, we grew the broth on two kanamycin plates. We picked two of the colonies and extracted the DNA and performed a plasmid prep by centrifuging the cells with three

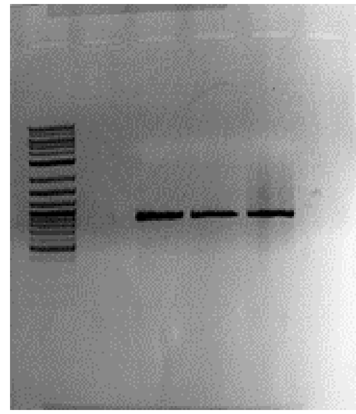

Figure 2 *- Each of the three PCR samples align with the 1200 base pair marker, confirming that the PCR of Bla534 was successful*

Figure 4 *- Results from the Gel Electrophoresis of the plasmids from the transformed* E. coli *cells. The results of the gel electrophoresis show that the plasmids did not take up the insert DNA, because the base pair number is lower than it should be.*

solutions, I, II, III, successively and then with ethanol. We then performed another gel electrophoresis to determine if our gene was successfully inserted into the plasmid.

Results

Results from the Gel Electrophoresis of our PCR Products of Bla63 and Bla534 were successful (Figures 1 and 2). There was also successful Bla63 colony that was grown Figure 3a and 3b). However, gel electrophoresis showed that the plasmids did not take up the insert DNA during transformation of Bla63 (Figure 4).

Discussion

The purpose of our research is to determine if our genes from *E. meningoseptica* (Blab534 and g63) are beta lactamase genes. Our research is still in need of continuation. Due to faulty DNA vectors, and products being lost when our lab location changed, our research was delayed. However, with continued research, we will be able to determine if our putative beta lactamase genes are actually beta lactamase genes. If we are able to obtain colonies of successfully transformed *E. coli* that contain plasmids with inserted DNA, we will be able to send in the DNA for sequencing to test the putative beta lactamase genes. If the sequencing confirms our results, we will be able to test these genes for antibiotic resistance against multiple antibiotics, furthering our knowledge on the antibiotic resistant qualities of our genes.

References

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