Testing antibiotic resistance in a segment of DNA from Elizabethkingia miricola

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ABSTRACT

As a class, our goal was to determine if a certain gene in *Elizibethkingia miricola* is an antibiotic resistance gene. Through computer analysis, we were able to determine many possible beta lactamase resistance genes. We tested these genes through a polymerase chain reaction. We made millions of clones of our gene and by doing so, we were able to insert that gene into *E. coli* cells and test whether or not it was a gene that caused beta lactam resistance. Our gene was proven through this to be a beta lactamase gene, which coded for beta lactam resistance in *Elizibethkingia miricola.*

INTRODUCTION

Elizibethkingia miricola is a type of bacterium discovered by Elizabeth O. King in 1960 (King 1959). She was studying meningitis in pediatric patients, more specifically, the bacteria that cause it. There are three types of *Elizabethkingia* bacteria, two of which have known antibiotic resistance genes (Tak 2013). This type of bacteria is known to have antibiotic resistance to beta-lactam antibiotics. What is not known, is what genes cause this beta lactamase resistance in this bacteria. The goal of this study is to isolate certain genes that are thought to be beta lactamase resistance genes and implant them into *E. coli* in order to test the specific gene for beta lactam resistance.

MATERIALS AND METHODS

We tested to see if our sequence of Beta Lactamase was anti-biotic resistant. We conducted the experiment in class and in laboratory on the third floor of the Noble Research Center. We designed primers to the beta lactamase gene. Our primers were ATGTCAGGTAAAGCACAAAATTTACAGTCCATTTCGTGTTTTAA- as our forward primer and ACCGGCGAAATGAAATGGTAATTACCATTTCATTTCGCCGGT as our reverse primer. Next we set up the pcr reactions. Then we used agarose gel electrophoresis to determine if the PCR worked. We then set up ligation reactions of our PCR product with the vector DNA. We heat shock transformation of ligation mixtures into E. coli cells. The procedure we did with transforming of E.coli cell was first we obtained 20 µl of competent E.coli cells. Secondly we added 2 µl of our ligation mixture, then we "flicked" 3x to mix. We placed our solution on ice for 15-45 min. After sitting on ice we placed in a water bath that was at 42 degrees Celsius for 30 seconds and returned back to the ice. Finally we added 200 µl of recovery broth and turned in our solution to the TAs. After doing the transformation we counted the number of colonies on the plate. Then with assistance from Dr. Matts true recombination clones were identified by screening individual colonies for the PCR product. Our clone was tested for beta-lactamase activity by qualitative colorimetric assay using nitrocefin saturated disks.

RESULTS

We tested E.mir_ATCC33958_bla469, a specific gene of *Elizabethkingia miricola,* to see if it was beta lactamase resistant. Figures 1 – 7 show setting up to check for PCR products by using agarose gel electrophoresis. Figure 8 shows the results of the agarose gel electrophoresis. Figures 9 & 10 show setting up DNA ligation of putting our DNA clones into the E. coli vector. Figure 11 shows the nitrocefin-saturated disk. Since the disk has a reddish tint to it, there was proof of some beta lactamase activity meaning that E.mir_ATCC33958_bla469 is not beta lactamase resistant.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

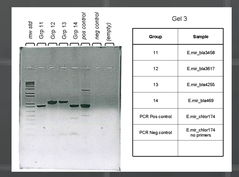


Fig. 9

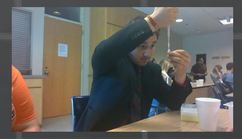
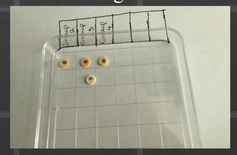


Fig. 10

Fig. 11



DISCUSSION

We know through prior research that Elizabethkingia anophelis and Elizabethkingia meningoseptica contain antibiotic resistant genes. Through this research tried to find which genes in Elizabethkingia miricola were resistant to beta lactamase. We found through our research that E.mir_ATCC33958_bla469 is not beta lactamase resistant. Our nitrocefin-saturated disk has a little red in it. Based on our negative and positive controls, the little bit of red means that there is a little bit of beta lactamase activity on the nitrocefin- saturated disk; therefore, the gene, E.mir_ATCC33958_bla469, is not beta lactamase resistant. Our nitrocefin-saturated disk only had a little bit of red in it. This indicates little beta lactamase presence. This could mean either one of two things. This could mean that the pictures and results were taken before the beta lactamase had time to set in or, that the gene, E.mir_ATCC33958_bla469, is close to being resistant to beta lactamase. That means that the gene is susceptible other antibiotics and is not antibiotic resistant. When fighting disease with antibiotics it is good to know whether they will be antibiotic resistant or not. This experiment narrowed down which genes are antibiotic resistant. Now that we know which ones are antibiotic resistant, we can do further tests to try to find which the way to make antibiotics that those genes cannot resist. If we were able to that we wouldn't have to worry about antibiotic resistance. In our experiment we showed that E.mir_ATCC33958_bla469, is not resistant to beta lactamase.

REFERENCES

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