Go Beta or Go Home: The Resistance of the Beta-Lactamase in the *Elizabethkingia anophelis* Bacteria

GRP#18
Section#002

AUTHORS' NAMES

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ABSTRACT

The purpose of this study was to determine which genes in the *Elizabethkingia anophelis* bacteria demonstrated beta-lactamase activity. It was hypothesized that our gene was positive for beta-lactamase activity. We took a beta-lactamase gene of the *Elizabethkingia anophelis* bacteria and cloned and a PCR reaction was set up. Next a DNA Ligation was set up to lock the DNA strand into a vector, and then heat shock transformation was used to trap the DNA into an *E-coli* cell. The bacteria was spread on a petri dish and colonies were grown and counted. Single colonies were then picked from the petri dish from which plasmid DNA was extracted and sequenced to confirm our construct. The clones were then screened and placed on a disk with nitrocefin to determine if our gene demonstrated beta-lactamase activity. Our gene was confirmed to be positive.

INTRODUCTION

As the earth's population increases, so do the number of the world's diseases. Each year, new sicknesses make their rounds in schools and offices, only to morph slightly and come back next year in a new form. This is why antibiotic resistance is so important in today's society. How can we be expected to combat an illness if it resists every antibiotic treatment we have used in the past? Testing bacteria for antibiotic resistance puts us one step ahead by eliminating potential cures that will ultimately fail instead of prescribing those antibiotics only to find out two weeks later that they were not effective. Infections from *Elizabethkingia anophelis* bacteria, as well as cases of the West Nile Virus, are on the rise, and both are linked to mosquitoes. Due to its resistance to many medicines, it is thought that *E. anophelis* may have β-lactamase genes (1,3). We hypothesize the *Elizabethkingia anophelis* bacteria we are testing contains a β-lactamase gene because it displays similar behaviors to other *Elizabethkingia* bacteria that exhibit antibiotic resistance. Previous studies have shown support for our hypothesis by stating that each of the *Elizabethkingia* species contains putative β-lactamase genes (2).

MATERIALS AND METHODS

The forward and reverse primer for a beta-lactamase gene of the *Elizabethkingia anophelis* bacteria were designed. A PCR reaction was then set up and Agarose gel Electrophoresis was used to identify our PCR product. Next a DNA Ligation was set up to lock the DNA strand into a vector, then heat shock transformation was used to trap the DNA into an *E-coli* cell. The bacteria was spread on a petri dish and colonies were grown and counted. Single colonies were then picked from the petri dish from which plasmid DNA was extracted and sequenced to confirm our construct. A single colony with our confirmed construct was then grown up and placed on a disk with nitrocefin on it to determine if our gene demonstrated beta-lactamase activity.

RESULTS

In the beginning of our experiment we were able to find the forward and reverse primers. In result of this we came up with:

Forward Primer: ATGTCAGGTAAAGCACACAAAATTTAAAAC

Reverse Primer: CCGGCGAAATGAAATGGTAA

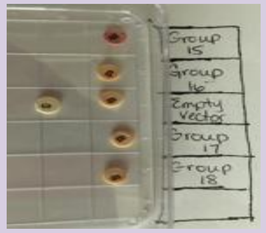
AATGGTAAAGTAAAGCGGCC
TTACCATTTCATTTCGCCGG

Our PCR reaction was then ready to start. Our PCR showed a product and our gel electrophoresis predicted weight was 840 and our band showed to be between 650- 850, which was very close to 840, therefore our result was confirmed. Furthermore, the ligation reaction was set up.

Heat opens up the E. coli cell and allows the DNA to slip in, then the cold traps the DNA in the cell. After we placed it on the Nitrocefin plate and waited for a certain amount of time we determined that it was positive.

Plate number 1: 43 colonies

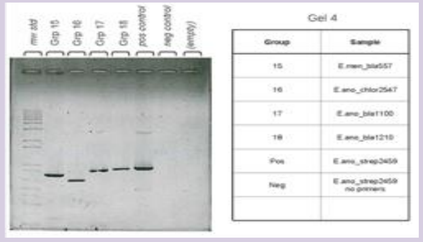
Plate number 2: 46 colonies



Group 18 Nitrocefin Results

Pipette and Test Tube for PCR and DNA Ligation

Colonies On the Petri-Dishes



Agarose Gel Electrophoresis Results

DISCUSSION

At the beginning of the semester, we were given the Beta-Lactamase gene. We designed te forward and reverse primers of the gene to move on to the PCR for the following weeks. We used a micropipette to add the required amounts of the respective agents to prepare for the Polymerase Chain Reaction. The PCR took place outside of class without our direct assistance. Our PCR showed a product and our gel electrophoresis, our predicted weight was 840 base pairs, and our band showed to be between 650 and 850 base pairs; therefore, we trusted our result. Because of this, our forward and reverse primer worked, and we had our DNA sequence. Using a P20 micropipette and disposable tips, we added deionized water, 10X Ligase buffer, Linear Plasmid Vector, PCR product, T4 DNA Ligase Enzyme. The ligation reaction was completed overnight. The DNA combined with the Vector awaiting the cloning process. We sequenced the DNA of the gene so that we could identify and predict the enzymes, traits, genotypes, and phenotypes. Both plates one and two contained colonies, meaning the transformation was successful. Finally, we examined the DNA sequence results for the detectable antibiotic resistance gene sequence. We observed the color change of the disk. The disk turned slightly red; therefore, it was considered positive, and our hypothesis was supported.

REFERENCES

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3. Teo J, Tan SY-Y, Liu Y, et al. Comparative Genomic Analysis of Malaria Mosquito Vector-Associated Novel Pathogen *Elizabethkingia anophelis*. *Genome Biology and Evolution*. 2014;6(5):1158-1165.