β-lactamase Gene Sequencing to Determine Antibiotic Resistance of Elizabethkingia Anophelis

GRP# 21
Section# 002 (702)

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ABSTRACT:

Antibiotics are used to treat infections caused by bacteria in the body. However many species are antibiotic resistant like *Elizabethkingia anophelis*. In our research we wanted to determine which antibiotics it was resistant to. We sequenced the genomic DNA; then we purified the DNA using organic extractions. We also needed to use PCR amplification by designing the primers so we could order them. We separated them by using a DNA agarose gel electrophoresis to separate by size. We then took a look at our ligation reactions: They seemed to be successful. Lastly we use heat shock transformation, to determine our results.

INTRODUCTION:

There are three different subcategories of *Elizabethkingia*; *E. meningoseptica*, *E. miricola*, *and E. anophelis*. *Elizabethkingia Anophelis* is a recently discovered bacteria found specifically in the gut of the *Anopheles gambiae* mosquito and it causes human disease. This is the bacteria that was the focus of our research. The reason behind looking at this bacteria is its resistance to B-lactam antibiotics. After extracting DNA from the bacteria, we sequenced the *E. anophelis* genomic DNA, cloned the B-lactam DNA through PCR Amplification, put the sample into *E. Coli*, and finally found out if it was resistant to the antibiotics or not through observing the results found after smearing it on the petri dish.

MATERIALS:

- 2.90µg of DNA
- .6µg forward and reverse primers
- 200µg of PCR nucleotides
- 1.75mm $MgCl_2$
- 2.5 units of Taq polymerase
- Thermocycler
- Agarose gel
- DNA vector
- 20µL Ligase
- 20µL of E. coli cells
- 200µL recovery broth
- 2 Agar plates

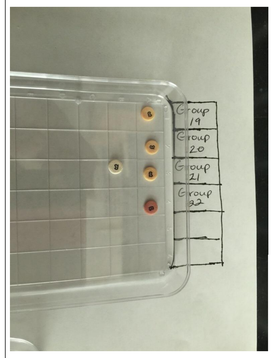
METHODS:

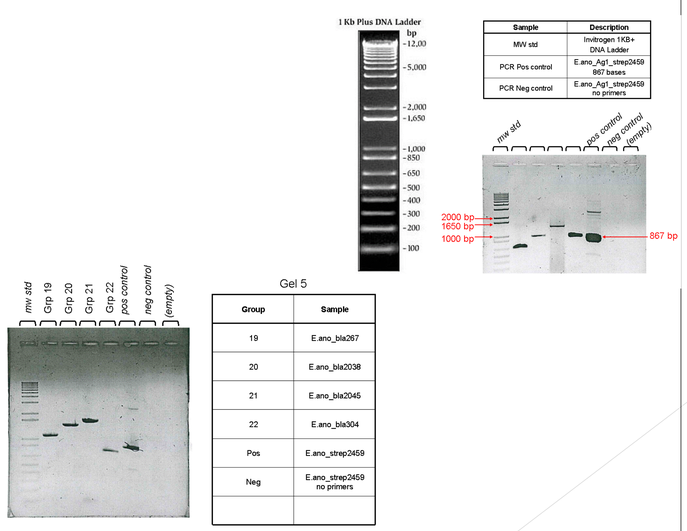
We first began by creating the forward and reverse primers by going on to www.idtdna.com, and had them created and shipped to our lab. The second step was to add the necessary to do a PCR reaction. After the ingredients were mixed together they were put into a thermocycler. The thermocycler went through 30 cycles of denaturation, annealing, and extension. On the final cycle the time of extension was extended to make sure the DNA was bound together, and then they were stored at 4°C overnight. Step three was preforming agaros gel electrophoresis. We placed the agaros gel, with our DNA inside, in a container filled with water and ran a charge through it. The charged particles allowed the DNA to move through the gel, and we can see where the dyed sample of DNA traveled through the gel. The DNA travels according to size so the farther it went the bigger the strand of DNA, and it can roughly tell us the amount of bases that we have in our DNA. Step number four was to remove the possible β-lactamase gene and place it into a vector. The vector was held together by Ligase, which is like a molecular glue. The fifth step was to attempt to get the vector with our possible β-lactam gene and allow it to penetrate inside of a cell. To do this we had to cool the E. coli cells for twenty minutes, and then place them into a water bath at 42°C, for thirty seconds, so that the cells would become pores, and allow the vector to pass inside of it. The cells were then returned to ice for three minutes. We then added recovery broth to the fragile cells, and they sat for one hour at 37°C. After one hour they were placed on an agar plate, which was coated in kanamycin, so that colonies could begin to grow. The next step was to count the colonies that resulted from our transformation that would hold a kanamycin resistance. The final step was to determine the β-lactam activity with a qualitative colorimetric assay by using nitrocefin saturated disks.

RESULTS:

The forward and reverse primers were created and then developed by a third party company. The forward and reverse primers is as follows:
(forward) ATGAAAAATAAATTCTCTTTATTC
(reverse) TTACTTTCTTTTCAGGTCCAGATC

The development of these primers enabled PCR amplification by providing a template for the copying of the Beta-Lactamase gene. The Taq-polymerase gene and the forward and reverse primers created billions of copies of the Beta-Lactamase gene thus preparing the gene for further experimentation. The genes that resulted from the PCR amplification needed to be checked in order to insure that the correct gene was cloned. This was done by gel-electrophoresis. The gene that our group cloned contained 1550 bases. The DNA strand was subjected to a positive charge and then pulled through the agarose gel until it could travel no farther. Based upon the stopping point of the strand of DNA, the size of the DNA strand was estimated to be 1550 bases. This verified that the correct gene was cloned and enabled further experimentation. The Beta-Lactamase then underwent a DNA litigation process. This process of the ligation of PCR product created a plasmid vector for molecular cloning. After undergoing DNA litigation, the plasmid vector underwent heat shot transformation and changed from DNA to E. Coli. The E. Coli was then grown in a petri dish and then the amount of colonies were counted. Dr. Canaan as well as the TA's proceeded to select antibiotic resistance isolates for DNA sequence analysis. The DNA sequence results were then examined for detectable antibiotic resistance gene sequence. A qualitative calorimetric assay for the presence of Beta-Lactamase gene in Nitrocefin saturated disks was performed. If the disk turned red, then the gene was Beta-Lactamase. The Nitrocefin saturated disk turned red. Therefore, the gene was a Beta-Lactamase gene.





Sample	Description
MW std	Invitrogen 1KB+ DNA Ladder
PCR Pos control	E.ano_Ag1_strep2459 867 bases
PCR Neg control	E.ano_Ag1_strep2459 no primers

Gel 5

Group	Sample
19	E.ano_bla267
20	E.ano_bla2038
21	E.ano_bla2045
22	E.ano_bla304
Pos	E.ano_strep2459
Neg	E.ano_strep2459 no primers

DISCUSSION:

The purpose of this research was to find and identify certain Beta-Lactamase genes that demonstrated Antibiotic resistance. This was done by cloning the gene through PCR amplification and then by DNA litigation to enable the gene to be transferred into E. Coli, a usable state. Then, a disk saturated in Nitrocefin was created to identify if the Beta-Lactamase gene was antibiotic resistant. Since the pale yellow Nitrocefin soaked disk turned red when exposed to the Beta-Lactamase gene, the gene that we had was antibiotic resistant. The results of the experiment solidified our hypothesis that the gene was a Beta-lactamase gene. This is important to know because this means that this particular gene was resistant to bacteria and is composed of Beta-Lactamase enzymes. This furthers the connection between Beta-lactamase and antibiotic resitance. Which leads to the question of what further research can be done. When given the β-lactamase gene we were working to discover if it was antibiotic resistant. Now that we have done so, there is still more that can be done. Future research could work to understand the complexity of the antibiotic resistance, and they could attempt to develop new antibiotics that would kill certain bacteria. Antibiotic resistant bacteria are extremely dangerous, and they can even be lethal. That is why it is so incredibly important to continue to discover and identify other forms of the Beta-Lactamase gene.

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