The Sequencing of Elizabethkingia meningoseptica Species to Determine the Antibiotic Resistance to a Beta-Lactamase Gene

Taylor Langdon, Crystal James, Dakotah Thomas, Christyn Mask, Dr. Patricia Canaan, Dr. Jessica Matts, Shanell Shoop, Nathanial Torres

GRP#11
Section#001

ABSTRACT

The motivation of this research was to discover if the species that was provided would be considered a beta-lactamase, which provides resistance toward antibiotics (Canaan, 2015). Through a series of cloning and sequencing, we were able to move forward with our conclusion. Our procedures were all successful, so we were able to collect accurate results. The beta-lactamase gene was positive, providing sufficient evidence that our beta-lactamase gene was antibiotic resistant to the species Elizabethkingia meningoseptica.

INTRODUCTION

This study derived from the idea that some bacteria is resistant to antibiotics, which are used to tend to bacterial viruses (Canaan, 2015). They can do this in one of four ways: demolish, avoid, alter, or jet, from the cell, the antibiotic (Canaan, 2015). We are most interested in the *Elizabethkingia meningoseptica*, which can cause disease in humans (Canaan, 2015). Essentially, the purpose was to expose whether our bacteria was antibiotic resistant or not. If we carry out the procedures provided in the materials and methods section, we hypothesize that our species will be a beta-lactamase gene.

MATERIALS AND METHODS

First, we listened to the background information the professor provided for the Elizabethkingia meningoseptica species that we experimented on. The species is found in nature and causes human disease. This species is resistant to more than twenty antibiotics including Amikacin, Spectinomycin and Oxacillin. The species Elizabethkingia meningoseptica have several different beta-lactamase genes which also means that the species makes more than one beta-lactamase protein.

The first step is sequencing the genomic DNA through DNA polymerase. By using the Polymerase Chain Reaction, we were able to amplify a single copy of the segment in our assigned DNA into billions of copies. Inside our segment of DNA is Taq polymerase, nucleotides and primers. The Taq polymeras and nucleotides were purchased, the DNA was extracted and purified this past summer and the primers were what we designed and ordered.

We distributed 10 ul of nucleotides into a small test tube that already had 70 ul of water in it using a micropipeter. We also placed 1 ul of taq polymerase, 2 ul of R-primers, 2 ul of F-primer, 10 ul of Taq buffer, and 5 ul of E. miricola DNA in the test tube. We placed the substances into the test tube after we determined the primer sequences the week before. Outside of class, the professor and helpers went through the different cycles of polymerize DNA and denature DNA. One cycle of the PCR begins by heating the reaction mixture to 95 degrees Celsius.

Then the temperature is reduced to 60 degrees Celsius so the primers will make hydrogen bonds. Then the temperature was raised to 72 degrees Celsius so the Taq Polymerase could add nucleotides to the three prime end of the DNA strand. After the first cycle there were two copies of the Target DNA sequence, and after the second cycle there were four copies of the Target DNA sequence.

This process helped us predict the beta-lactamase gene that may exist by testing our primer sequence and finding the start and stop codon in our DNA. Then we could predict our mRNA and protein. The PCR amplification of the predicted beta-lactamase gene helped us predict if it was beta-lactamase. We placed the gene in an antibiotic sensitive bacterium to test the clone for antibiotic resistance.

The next step is extracting the DNA from the sample of interest. We placed our copied DNA in gel electrophoresis so we could see the DNA separated by size. Our objective was to have our DNA sequence go through ligation so the DNA could encode the protein. We added 8 um of the PCR mix to 2 ul of blue dye and then placed the 10 um into one well.

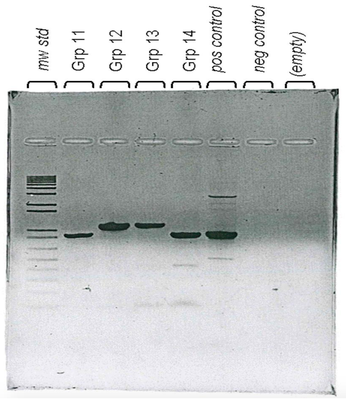
Next, we set up the ligation reaction in order to see what happens to our DNA after being placed in cold temperatures. We placed 2 ul of a Ligase buffer into a test tube that already had 11 ul of water in it. We also placed 2 ul of linear plasmid vector, 4 ul of PCR product and 1 ul of T4 DNA ligase Enzyme inside the test tube using a micropipette. After completing this, we kept the one test tube on ice and turned it into the professor. Outside of class, the professor and helpers incubated each ligation at 16 degrees Celsius overnight and then stored our samples in a refrigerator at 4 degrees Celsius until our next class.

Then we took the mixture we made the week before and put the mixture into an E. Coli strand that has zero resistance to antibiotics. We also used heat shock transformation of the E. Coli. We obtained 20 ul of competent E. Coli cells and then added 2 ul of your ligation mixture. We then flicked the mix three times and immediately placed the mix on ice for 15-45 minutes. Then we heat shocked at 42C in a water bath for 30 seconds. We then returned to ice for 3 minutes and also added 200 ul recovery broth at room temperature. The TAs incubated the samples at 37 Celsius for one hour after the class. They then placed plate aliquots on kanamycin agar plates. After, they incubated at 37 Celsius overnight and then parafilmed and transferred plates to a refrigerator for storage.

Finally, we went through DNA sequence analysis to check if we did the cloning of our gene correctly. We did this by counting our colonies in each dish. Out of the classroom, the TAs isolated and examined the plasmids and also screened for clones by Agarose Gel Electrophoresis. Next, we took the clones and screened them for beta-lactamase activity.

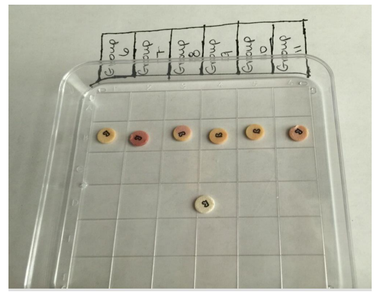
RESULTS

Throughout the experiment, we had one goal and that was to determine whether or not the DNA we had ben given included a beta-lactamase gene. First off, we covered background and the procedures necessary to begin the process of determining whether or not it is a beta-lactamase gene. We started with a strand of DNA and were able to identify the forward and reverse primer of the given strand. After ordering the reverse and forward primers, we combined the Taq Polymerase, the gDNA, forward primer, reverse primer, all 4 dNTPs and a buffer all in the same tube using a micropipetter. We then placed the tube into a Thermocycler for 30 cycles to generate PCR. After the mixture underwent PCR, we found that we were successful in copying the DNA. Our next step was to add the proper dye/gel to make it visible and distribute it into the wells to determine the size, ass seen in picture 1. As seen in picture 2, our gene did read and ended up falling to about 837 bp. We were now able to jumpstart the ligation process, adding together 10x ligase buffer, linear plasmid vector, our PCR product from before, and T4 DNA Ligase Enzyme to dH2O. After many other processes and steps, we were able to determine if our gene was in fact a beta-lactamase gene or not. It resulted in coloration so our gene is concluded to be a beta-lactamase gene.



Gel 3

Group	Sample
11	E.mir_bla3458
12	E.mir_bla3617
13	E.mir_bla4255
14	E.mir_bla469
PCR Pos control	E.mir_chlor174
PCR Neg control	E.mir_chlor174 no primers



DISCUSSION

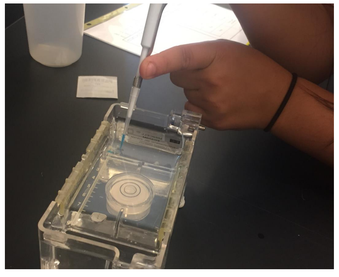
The PCR Product for Group 11 is positive, showing that all primers were correct. To study results all we have to do is change the primers and see how it affects our results.

In our experiment we tried to figure out which gene would breakdown hydrocarbons in oil. To do this we filled in DNA sequence gaps to produce a sequence to help the breakdown process. While working on our DNA agarose gel electrophoresis we created billions of copies of PCR. Then, after some preparation we were able to use a heat shock to transform our E-coli bacteria.

After taking clones and doing our beta-lactamase screening we were able to see our results. Our beta-lactamase genes in the Elizabethkingia species showed that Group 11's nitrocefin saturated disk had a positive reaction.

Group member uses a micropipette to mix substances together.

Group member uses a micropipette to place their copied DNA into a well in Gel Electrophoresis.



REFERENCES

Canaan, P., personal communication, 2015