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, Nathaniel Torres

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 infections. The motivation of this research was to discover if the species that was provided would be considered a beta-lactamase gene. First, we listened to the background information the professor provided for the Elizabethkingia meningoseptica species that we experiment on. The species is found in nature and causes human disease. This species is resistant to more than two 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. Using the Polymerase Chain Reaction, we were able to amplify a single copy of the segment from our assigned DNA into billions of copies. 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.

MATERIALS AND METHODS

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 for antibiotic resistance.

The next step is extracting the DNA from the sample of interest. We placed our PCR product from the sample into a Thermocycler for 30 cycles to generate PCR. This process helped us predict the beta-lactamase gene or not. It resulted in coloration so our gene is concluded to be a beta-lactamase gene.

RESULTS

Throughout the experiment, we had one goal and that was to determine whether or not the DNA we had been 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 microappetite. Then we placed the tube into a Thermostycycler 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, as seen in picture 1. As seen in picture 2, our gene did read and ended up falling about to 837 bp. We were now able to jumpstart the ligation process, adding together 10x ligation 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.

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 the 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.