β-lactamase Gene Sequencing to Determine Antibiotic Resistance of *Elizabethkingia Anophelis* in the Gene AG1-BL 1516

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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 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. Then we purified the DNA using organic extractions. We then took a look at our ligation reactions: they seemed to be successful. Lastly, we use heat shock transformation to solidify our results, and plated them on an agar plate containing kanamycin.

Keywords: *Elizabethkingia anopheles*, Beta-lactamase, Antibiotic resistance

Introduction

There are three different species of *Elizabethkingia*: *E. meningoseptica*, *E. miricola*, and *E. anophelis*. *E. anophelis* is a recently discovered bacteria found specifically in the gut of the *Anopheles gambiae* mosquito and was found to cause human disease. This is the bacterium that was the focus of our research. The reason behind looking at this bacterium is its resistance to ß-lactam antibiotics. We have determined that there are multiple strands of beta-lactamase genes that are resistant some type of antibiotic. With multiple genes resistant to multiple antibiotics, a need to test each potential strand arises. With this being the case, we must first extract the desired DNA from the bacteria. After extracting DNA from the bacteria, we sequenced individual strands of *E. anophelis* genomic DNA, cloned the β-lactam DNA through PCR Amplification, inserted the sample into *Escherichia coli* cells, and finally found out if the specific DNA strand was resistant to a weak antibiotic or not through observing the results found after smearing it on the agar plate.

Methods

To begin the experimental process, we multiplied the strands of AG1-BL1516 using a polymerase chain reaction. Following this step, we took the PCR product and tested it in an agarose gel using the electrophoresis process to assure that the PCR reaction occurred, and that the number of bases was correct. After this was complete, we purified the PCR product by adding water and 0.5 mL of phenol: chloroform isoamyl alcohol, an organic substance, in order to create a product containing only the gene we amplified. Another organic substance, chloroform: isopropyl alcohol was also added for the same cleanup procedure to occur. After they were added, incubated, and spun down, the liquids were removed from the product. Next, the enzymes Nhel-HF and SaCl-HF were added to the product and left in a hot bath to incubate the reaction. The cleanup process was then repeated with both phenol: choloform isoamyl alcohol, and choloform: isopropyl alcohol added and removed after an incubation and spin down process. Following the second cleanup step, we put the product through a ligation process where all liquid is removed from the product, and a ligase buffer, digested vector, and DNA ligase are mixed with the digested PCR product and left in a warm bath, then cooled down after assuring that the reaction occurred. Finally, we reach the transformation process where we mix competent *E. coli* cells with the ligation product. The mixture is incubated on ice, then briefly placed into hot water in order to heat shock the cells. Once placed in hot water, they open up in order to take in the DNA. Immediately after the heat shock, they are placed back in ice and left for two minutes. They are then incubated in warm water for over one hour and plated on agar plates containing kanamycin in order to kill the cells without the beta lactam DNA.

Progress to Date

We have completed all steps explained in the methods section, and at this point in our research, we expect colonies to form on the kanamycin agar

plate. However, in this particular experiment with the AG1-BL1516 strand, no formation occurred after incubation. After determining that colonization did not occur, the process was redone starting with the phenol: chloroform isoamyl alchol cleanup, and completed all the way through transformation and plating of cells. Again no results were produced, and no colonies grew. This is a direct result of one of two possibilities: the strand is not resistant to antibiotics, or an error occurred in the process, most likely in the digestion or ligation steps of the experiment.

Discussion

The purpose of this research was to identify and confirm putative beta-lactamase genes in *E. anopheles* Ag1 bacterial strain. This was done by the processes explained earlier up to transformation and the plating of the cells. If the transformation is a success, the next step will be to screen the bacterial colonies to find the one that has the putative beta-lactamase. Our colonies, once plated, did not grow which leads us to believe that there was either a problem at the digestion or ligation stage of the project, or that the beta-lactamase gene is not antibiotic resistant. Along with this particular strand, many others were being tested at the same time by other experimenters, with minimal success. Though we believe each method up until the plating of the bacteria was done correctly, a test of the digested PCR product showed no sign of PCR. This led to the belief that the strand's resistance was not the issue, but rather that some sort of contamination occurred in the later steps of the experiment. Though multiple attempts were made to correct contamination, no solution was found prior to the deadline for information to be submitted. In the future, the putative beta-lactamase still needs to be cloned and tested to see if they are indeed beta-lactamases or some other protease. To determine if they are beta-lactamases or not, once cloned (and sequence confirmed), we will do a Kirby-Bauer and minimal inhibitory concentration assays. If they are beta-lactamases we will be able to determine what beta-lactam antibiotics they are either sensitive or resistant too. 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-lactam antibiotics that can inhibit these bacteria.

References

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