Determining that the E.ano_Ag1_bla267 gene in *Elizabethkingia anophelis* bacteria codes for Beta-lactamase

GROUP 19
Section 002

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ABSTRACT

The focus of this experiment was to identify beta-lactamase-coding genes in *Elizabethkingia anophelis* bacteria. We were presented with the gene E.ano_Ag1_bla267 that had indicators that it might be a beta-lactamase gene. To determine if this gene is a true beta-lactamase gene, we conducted PCR, inserted the amplified *E. anophelis* DNA into vectors, inserted the resulting vectors into *E. coli*, and identified true recombinant clones and tested them for beta-lactamase activity. We found that our gene is a strong positive. In conclusion, we determined that the E.ano_Ag1_bla267 is a true beta-lactamase-coding gene.

INTRODUCTION

Elizabethkingia anophelis is a type of bacteria that is found in the gut of malaria-carrying mosquitoes and is known to be resistant to a large number of antibiotics (Kämpfer et al. 2011). Beta-lactams are a large class of antibiotics that are characterized by a β-lactam ring. Beta-lactamase is an enzyme that hydrolyzes and breaks the ring thus disabling the antibiotic and saving the bacteria (Ambler 1980). Before 2015, researchers knew that *E. anophelis* had antibiotic resistance genes due its immunity to a large number of antibiotics, but did not know what genes coded for beta-lactamase. During the summer of 2015, Dr. Patricia Canaan identified twenty possible beta-lactamase-coding genes. In our experiment, we took a potential beta-lactamase-coding gene, E.ano_Ag1_bla267, and confirmed that it is a true coder.

MATERIALS AND METHODS

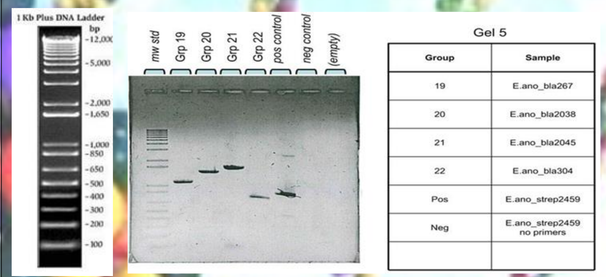
First, we designed forward and reverse primers to conduct PCR amplification. This process allowed us to work with millions of copies of our possible beta-lactamase-coding gene. Next, to determine if our PCR was successful, we counted the number of base pairs using agarose gel electrophoresis. After the gel was run, we compared the known number of bases of the gene to the number that resulted from the electrophoresis.
Next, we inserted our gene's DNA into pSKB3 vectors through a ligation process. After this was completed, we conducted a heat shock transformation of the ligation mixtures into *E. coli* cells. Then, after allowing time for the resulting colonies to grow, we checked the plates for transformants and identified true recombinant clones. Last, using qualitative calorimetric assay with nitrocefin saturated disks, we will determine if our gene is a true beta-lactamase coder. If the disk turns red upon contact with our gene, then the gene is a confirmed beta-lactamase gene and if it stays a pale yellow, it is not.

RESULTS

To begin, primers had to be designed in order for the gene, which was 1,065 base pairs long, to be replicated.

Forward Primer 5'- ATGAAAAACGTATTTGATATAC
Forward Compliment: TACTTTTTGCATAAACTATATG
Reverse Primer: 5'- CCCCCGAACATTATATTCATAA
Reverse Compliment: TTATGAATATAATGTTCGGGGG

After designing and receiving the primers, our group received a successful result from our PCR amplification of the gene. Agarose gel electrophoresis helped confirm that the PCR reaction was a success, as seen in the gel below. (Figure 1)



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

Figure 1: Group 19
The images show the whether the reactions were successful or not. If the reaction was a success, the DNA will be visible in the gel, like the examples in the photo.

Using a Ligation reaction, we were then able to create a recombinant vector containing our gene which we could insert into E. coli cells through the process of heat shock (Rapid heating and cooling of the cells). This process resulted in our E. coli samples successfully accepting the vector and taking the genes traits. Growing the colonies on an augar plate that was infused with Kanamycin prevented any cells that didn't accept the vector from growing (Figure 2). The colonies that did grow were harvested and tested for resistance to beta-lactam antibiotics. Analyzing the Nitrocefin Qualitative Colorimetric Assay (Figure 3) we determined that the gene was in fact a beta-lactamase.

Figure 2: Group 19
The images depict our augar plates after allowing the colonies to grow. The left plate had approximately 900 colonies while the right plate had approximately 940 colonies. The 1840 colonies counted accepted the vector.

Figure 3: Group 19
The image depicts the plates of colonies and the results of the final test for beta-lactam resistance. The negative control disk is white, indicating no resistance. The disks that turn red indicate that those genes have a general resistance to beta-lactam antibiotics.

DISCUSSION

An alternative to cloning, polymerase chain reaction (PCR) can be used to directly amplify rare specific DNA sequences in a complex mixture when the ends of the sequence are known. We took our PCR amplification of our predicated beta-lactamase coding gene and cloned it into a vector. We examined our cloned beta-lactamase gene's DNA sequence and designed a primer to amplify from start codon to the stop codon. The start and stop codon helped us find the forward and reverse primers and also their complementary primer. From our methods we could easily determine our needed information. We followed the instructions carefully and determined that forward primer is: ATGAAAAACGTATTTGATATAC. The reverse primer is: CCCCCGAACATTATATTCATAA. The reverse compliment is: TTATGAATATAATGTTCGGGGG which is exactly what the computer program told us when we checked our findings. Next, Dr. Canaan sent off our primers to the company IDT DNA so they could make/amplify these strands which we will use to start the PCR process. We started the PCR process by checking our PCR for products by agarose gel electrophoresis. We did this to determine the presence or absence of our PCR product and measure the length of the DNA molecule. Through electrophoresis, we have determined that we completed PCR correctly because our known amount of bases is very close to what we were able to measure, which was around 1100 bases. We had no ghost lines that we could observe which means the primers coded correctly. Because our PCR did yield the correct product, we can safely assume that we were able to prep the solution properly. Next, we used heat shot transformation to inset our DNA vectors into *E. coli*. After mixing the reactants, heating the DNA will denature and break the bonds. Then, reducing the temperature allows the primers to bind to the DNA and start the amplification process. After this process, we checked transformation plates for transformants. We counted approximately 900 colonies one plate and 940 in the other. After our clone was tested for beta-lactamase activity by qualitative calorimetric assay using nitrocefin saturated disks, our DNA results came back positive. In conclusion, we determined that our gene does, in fact, code for beta-lactamase and, therefore, codes for antibiotic resistance.

REFERENCES

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