To Be A Beta-Lactamase, Or To Not Be A Beta-Lactamase

GRP: 20
Section #2

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ABSTRACT

Elizabethkingia anophelis is a bacterium discovered in the gut of mosquitoes and is responsible for causing meningitis in infants in underdeveloped countries. This bacteria has an ability to confer antibiotic resistance to different classes of antibiotics. The enzyme responsible for this occurrence is a Beta-lactamase enzyme that hydrolyzes carbon bonds in antibiotics, thus breaking them apart. We investigated the presence of this Beta-lactamase and its true function through the use of DNA polymerase chain reaction (PCR), gel electrophoresis, and DNA ligation to insert the cloned sequence into *E.coli* for antibiotic screening. The results showed a positive confirmation of the possible Beta-lactamase, however the true strength of its antibiotic resistant properties was not fully discovered. Our research highlights the use of DNA ligation and Polymerase Chain Reaction to separate DNA strands and identify potential true Beta-lactamases.

INTRODUCTION

The strain of bacteria examined in this research is *Elizabethkingia anophelis. Elizabethkingia anophelis* is a recently discovered bacterium isolated from the midgut of the Anopheles gambiae mosquito in 2011 (Kampfer 2670). The recent discovery provides a need for research into the strain of bacteria as it has been shown to have health hazards. E. anophelis has recently been reported to cause neonatal meningitis in the Central African Republic (Lau 232). The main fear associated with this new bacterial strain is that it may be resistant to many types and classes of antibiotic. The mechanism responsible for antibiotic resistance is known as a Beta-lactamase.

A Beta-lactamase is an enzyme created by certain bacteria to provide resistance to Beta-lactam classes of antibiotics. Examples of certain classes of antibiotics are penicillin, carbapenem, and cephamycin. The beta-lactamase has a four carbon ring structure, which is identified as a β-lactam, plus different chains that alter the molecular structure. The direct opponent of this enzyme and the bacteria it bonds with is antibiotics; specifically β-lactam antibiotics. β-lactam antibiotics are a large class of antibiotics that include any individual with a β-lactam ring in its structure. The major classes include penicillin, cephalosporin, monobactam, and carbapenem. β-lactam antibiotics inhibit cell wall formation by not allowing the layers of the peptides to bond together in bacterial organisms and prevent the bacteria from forming a closed cell. In order for the Beta-lactamase to perform its function in stopping the antibiotic from entering the infected cell, a chemical reaction is produced. Through a process known as hydrolysis, the enzyme breaks apart a bond in the carbon ring, which deactivates the antibiotic's properties.

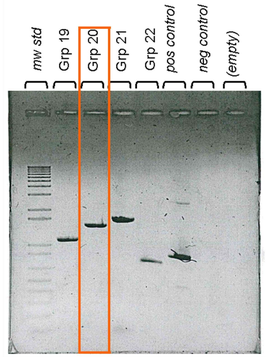
Previous studies have shown that by identifying possible Beta-lactamases, patients can be more effectively treated with antibiotics not of the ampicillin, carbapenum, or cephalosporin classes. The aim for this research is to identify if our sequence of *Elizabethkingia anophelis* can be identified as a true Beta-lactamase enzyme. Once it is identified, it can be sequenced and tested for what classes of antibiotics it is fully resistant to as well as susceptible.

METHODS AND MATERIALS

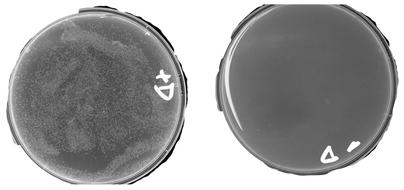
1. Sequenced genes to order for research
   A. Check accuracy on www.bioinformatics.org
2. Mixed all ingredients in test tube to for PCR Reaction
   A. Ingredients included: 70µl dH_2O; 10µl 10X Taq Buffer; 10µl 10X dNTP's; 5µl *E. anophelis* gDNA; 2µl F-Primer; 2µl R-Primer; 1µl Taq polymerase *ingredients were mixed using a micropipetter
   B. After mixing, the PCR reaction was immersed in 96ºC for 30 sec followed by 30 cycles of the following:
      Denaturation at 94ºC for 30 sec
      Annealing at 57ºC for 30 sec
      Extension at 72ºC for 1.5 min
      Concluding with a final extension at 10 min at 72ºC
      Store at 4ºC
3. Clone DNA- First Reaction of cloning. 1 beta lactamase gene
   A. Ingredients included: 2µl of dye and 8µl of PCR mixture. *ingredients were mixed using a micropipetter
   B. After mixing the dye, the mixture was put into the designated area in the Agarose Gel. The mixture and gel was then placed in a temperature controlled area.
4. Litigation of PCR product to plasmid vector for molecular cloning
   A.Ingredients included: 11µl dH_20; 2µl 10X Ligase buffer; 2µl Linear Plasmid Vector; 4µl PCR Product; 1µl T4 DNA Ligase Enzyme *ingredients were mixed using a micropipetter
   B.After mixing, the sample was incubated at 16°C overnight then was stored in the refrigerator at 4°C
5. Heat Shock Transformation of E. coli
   A. Ingredients: 20ml competent E. coli cells; 2ml ligation mixture
   B. After mixing with slight "flick" of tube, the mixture was put on ice for 15-45min. It was then heat shocked at 42°C for 30 sec and returned to ice for 3 min. 200ml of recovery broth at room temperature was then added.
   C. It was later incubated at 37°C for 1 hr, plate aliquots were put on kanamycin agar plates, it was incubated at 37°C overnight, and then the parafilm and transfer plates were stored in the refrigerator.
6. Counting Colonies
   A. After colonies were formed, a Sharpie was used to point out specific colonies on each plate
7. Checking Beta Lactamase Results
   A. Observed beta lactamase to see if it was a putative beta lactamase which would have the color tent of red

RESULTS

In order to begin the PRC process, both a forward and reverse primer were needed to be formed, so that we could properly clone the desired base paired fragment. It requires a start a codon of ATG and a stop codon of TTA. The recommended size is between 18 and 30 base pares. Our parings came in at 19 and 21 pairs. The base parings are as follows:
E_ano2038-Forward: ATGAAAAAAGTTATATTCC
E_ano2038-Reverse: TTATATTTTTTAATTCCTTC

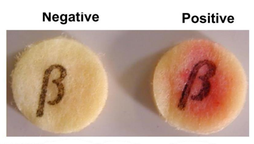


Our PCR is highlighted in orange, and the positive and negative controls are to the right. This process showed us that our PCR is between 1200 and 1500 base pares. Confirming that our base paring was accurate with 1371 parings. This allowed us to proceed to further testing.



The plate labeled +D is known as the positive control, and the plate labeled -D is the negative control. Our plates are below. Each colony is marked with a black dot. We ended up having ten colonies in all. The showing of colonies indicates that the ligation resulted in effective cloning and shows that we have the possible Beta-lactamase functioning properly

The left side represents our Beta-lactamase, and the right side represents the control groups. A negative control will result in a pale yellow; while a positive control will result in a red. Our Beta-lactamase resulted in a slight color change making it a possible true Beta-lactamase. The lack of substantial colonies provides a link to the reason for only a slight color change. Further DNA sequencing would be required to quantitatively determine the strength of the Beta-lactamase.



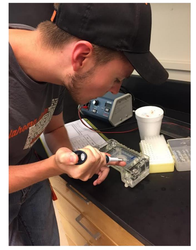
DISCUSSION

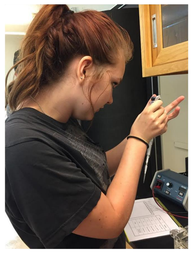
The results of our experiment yielded an overall positive finding. The beta-lactamase in question has enough evidence present to be conferred at slight resistance levels. Because the assay disk experienced only a slight color change, we did not experience a strong positive and therefore the Beta-lactamase needs further testing to ensure a positive identification.

Regardless of the slight color change in results, the research ran smoothly and we had very few complications. Of the ones we encountered none were severe. One error of ours was mixing up a letter in the reverse primer of our DNA sequence. However, due to using the bioinformatics system to check our primers we quickly identified and rectified the error. We also had difficulties putting the ligase enzyme into the pipeter, but we were able to release the ligase enzyme back into the solution tube and re-pipet the solution effectively on a second run through.

Our group used excellent teamwork throughout the entire experiment. We were hoping for an abundance of colonies after the heat shock process, but this did not happen. We ended up having only ten colonies all together. The reasons for this could be possible error in the ligation and the heat shock transformation could have encountered problems with timing and not allowing the cells to properly open or close too soon. The final phase was to test for the presence of the Beta-lactamase in the DNA assay that was created. By adding Nitrocefin which is a compound that can detect beta-lactamase enzymes via color change, we were able to quickly produce results. Due to time constraints in the course, the assay was not able to go for DNA sequencing which would have produced stronger and more definitive results.

Our results assist in the understanding of the molecular structures of antibiotic resistant enzymes. The Beta-lactamase enzyme is crucial in a bacterium's ability to neutralize the effects of different classes of antibiotics. By isolating possible Beta-lactamase genes, our research can be continued by testing different antibiotic classes and determining what can be effective in the fight against bacterial immunity. As for the current phase, a procedure involving PCR, gel electrophoresis, and DNA ligation to isolate a gene sequence, then inserting it into a vector for growth in *E. coli* cells is efficient. Not all Beta-lactamases will be present in the DNA for *Elizabethkingia anophelis*, however in our group's case, a putative lactamase was confirmed and can be used to test for more resistance in the future.





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