Determining the Authenticity of Possible Antibiotic Resistant Genes from the Bacteria *Elizabethkingia anophelis*

GRP#24
Section#2

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ABSTRACT

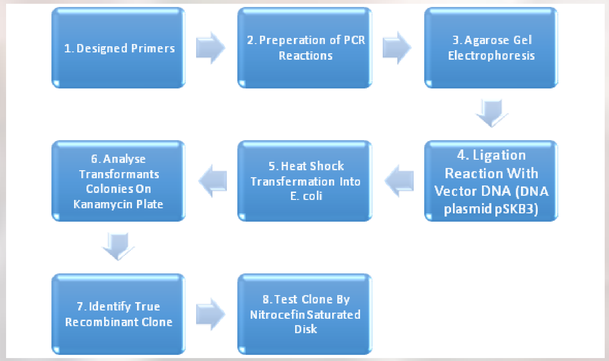
We hypothesize that the bacteria *Elizabethkingia anophelis* is able to produce 3,851 β -lactamase enzymes, and were assigned #3,289 to study. β-lactamase cleave β-lactam antibiotics such as Penicillins, Cephalosporins, and Carbapenems. To determine if E.ano_3289 is a β-lactamase enzyme or not we chose PCR amplification and molecular cloning to test our hypothesis. Using Nitrocefin as a β -lactam substrate, we observed it supported our hypothesis. We have demonstrated the *E. anophelis* is a true β-lactamase enzyme and warrants further investigation.

INTRODUCTION

According to our research, β-lactamase is an enzyme produced by various bacteria that provide a resistance to β-lactam antibiotics such as penicillins, cephamycins, and carapenems, although carapenems are relatively resistant to β-lactamase. β-lactams ranges of activity include easy penetration of gram-positive bacteria, while the outer cell membrane of gram-negative bacteria prevents diffusion of the drug. Since β-lactams have trouble entering human cell membranes, they are ineffective against atypical bacteria. Evidence shows that the gram-negative rod-shaped bacteria, Elizabethkingia, has several different β-lactam genes. Elizabethkingia is a pathogen which can survive in most conditions, including chlorine-treated water supplies. This pathogen colonizes on many different hospital tools and has become common for infection in hospital environments. This bacteria is able to resist a multitude of antibiotics by several different factors including pumping the antibiotic out of the cell, destroying it, modifying it, or dodging it.It can also build an immunity against them.

Elizabethkingia anophelis is responsible for the disease, meningitis, as well as pneumonia. Since these are fairly common diseases, an antibiotic is needed to treat them. The sole purpose of this experiment is to find the gene that codes for β-lactamase resistance in Elizabethkingia.

MATERIALS AND METHODS



RESULTS

For this experiment, our group (Group 24) was assigned a specific gene to test for the beta-lactamase activity that could possibly be present.

In order to test for the presence of beta-lactamase, we had to clone our assigned gene to possess a testable amount of DNA. To accomplish this, we coded starter proteins for PCR.

The start and finish primers that we determined were:
E_men3458-Forward: 5'- ATGAAAAGAATTAGTATTATTTTTCTG
E_men3458-Reverse: 5' - TTAGGGTTTATTTTTTTTATTTAAAGC

After receiving our ordered primers, the PCR amplification reaction was then performed allowing for our group to gain multiple copies of our desired gene. To begin the cloning process, we first mixed our PCR fragment with a DNA encoding protein and a plasmid vector that gave the cells a unique trait of Kanamycin resistance. Following, the T4 DNA ligase helped "glue" the protein, HIS-TAG, and vector together for experimentation. Once we received our product the following week, it was mixed and combined with experimental *E. coli* for testing of its properties. Once completed, the resultant bacteria was spread over a petri dish made with kanamycin, for the purpose of isolating only the *E. coli* cells that had successfully taken in the vectors. The vector-positive *E.coli* created colonies as can be seen in our results (Figure. 1).

Next, several colonies were isolated from the plates and screened for successful clones of our desired genetic sequence through putting the *E. coli* through agarose gel electrophoresis, the results of which can be seen and compared to the control (Figure. 2; Group 24 results, Figure. 3; Control for comparison). The results of the gel run confirm that our clones had successfully been made without any contamination present.

Once we received the confirmation that the clones were present and isolated, we proceeded to move to the screening process for the presence of beta-lactamase activity to conclude our experiment . This was accomplished through the use of a nitrocefin qualitative colorimetric assay, where our product was placed on a small disk that was soaked in a yellow nitrocefin compound. This yellow compound would convert to red-color with the presence of beta-lactamase activity. Our gene concluded to test positive for beta-lactamase activity when the disks changed color (Figure. 4). This conclusion confirmed our expectation that our gene was would test positive for beta-lactamase because of its genetic sequence.

Experimental error was avoided within our experiment through isolation and the double checking of our results through multiple testing processes. There is always a margin for human error however, because the results of the reaction are clear and our determinations are successfully accurate, error seems to be minimal. One of the strengths of our experimental design is that it is easily replicable and the methods are straightforward.

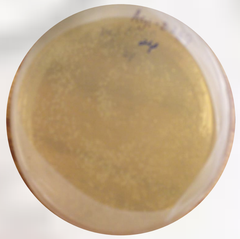


Figure. 1

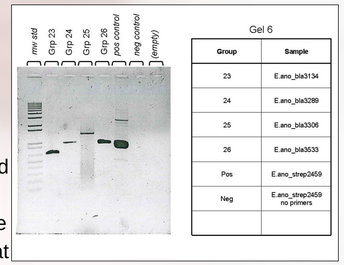


Figure. 2

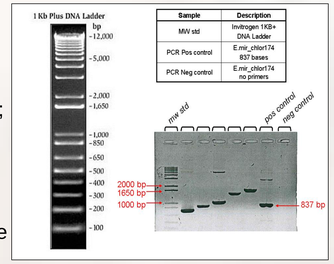


Figure. 3

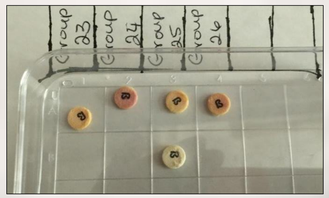


Figure 4

DISCUSSION

During this course experiment, the presence of an antibiotic resistant bacterial-enzyme was determined positive in an isolated section of chosen DNA (assigned to our group, group 24). To make this determination, a small section of DNA was extracted from a strain of given bacteria known as *Elizabethkingia sp*. Once the DNA of interest was isolated away from the entire strain (DNA sequence) it was then copied through a process of PCR (Polymerase Chain Reaction) in order to test for the presence of a specific antibiotic resistant enzyme known as beta-lactamase. The presence of this enzyme was confirmed positive, allowing for the possibility of further studies and tests to determine the strength of the resistance of this enzyme.

The DNA was extracted from a strain of *Elizabethkingia* because previous evidence had showed that there are several different beta-lactamase genes present in this bacteria. Therefore, it has been proven that the Elizabethkingia strain in question most-likely will produce more than just a single beta-lactamase protein. This previous evidence supported our hypothesis in predicting the presence of the beta-lactamase gene within our DNA sequence.

The purpose of this course experiment was to first amplify the extracted DNA in order to confirm and/or identify the presence of beta-lactamase in a section of given DNA sequences to then further test the antibiotic resistance of the enzyme in the future.

Given strains of *Elizabethkingia* bacterial-species were provided to the class for control and testing; each group was given a different DNA sequence in order identify if the chosen DNA section for our group had the presence of beta-lactamase or something else. With the extraction of the DNA of interest, the obtained samples of ruptured cells and purified DNA was closely analyzed. The extracted DNA was placed in a small tube of ruptured cells, detergent, glass beads and a vortex to isolate the section of interest and prepare it for cloning.

After the extraction process, it was possible to test for all existing genes. Once the antibiotic resistant genes were identified within the chosen DNA, the PCR amplification then was able to take place. This cloning process served the purpose of study accuracy with the chosen gene in question that needed to be further tested. Our DNA sequence was amplified through the process of PCR due to an interest in looking at one smaller region of the extracted DNA containing an important enzyme. This enzyme is known as Beta-lactamase, an enzyme (protein) secreted to digest the beta-lactam-type (resistant bacteria) antibiotics, which was confirmed to have positive activity within our experient.

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