The Positively Positive Control Group

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GRP #15 Wednesday Section

ABSTRACT

Elizabethkingia meningoseptica is a bacteria prevalent in nature. The *Elizabethkingia spp.* has two other discovered species: *E. anopheles,* and *E. miricola.* This genus of bacteria has been known to contribute to meningitis in humans. Little is known about the genome of this genus, and in this experiment we investigate which genes in *Elizabethkingia meningoseptica* contribute to antibiotic resistance. Through PCR, gel electrophoresis, DNA ligation and transformation we inserted a specific *E. meningoseptica* gene into *E. coli* and tested the bacteria for antibiotic resistance.

INTRODUCTION

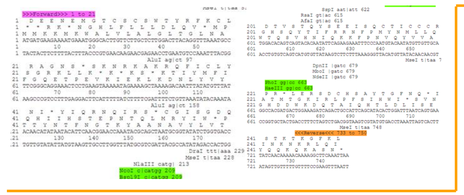
Elizabethkingea spp.—specifically *Elizabethkingia meningoseptica, Elizabethkingia anophelis,* and *Elizabethkingia miricola*—are a genus of bacteria that scientists first found in nature, and later in the gut of mosquitoes and isolated from the Mir space station (Matyi et al. 2015). Scientists believe that *Elizabethkingia spp.* can cause serious infections in humans (Li 2015). These bacteria are resistant to over 20 antibiotics that include tetracycline and β lactam based antibiotics such as semisynthetic penicillin. *Elizabethkingea spp.* have multiple genes that code for proteins that give the bacteria multiple different mechanisms to counteract different antibiotics. One mechanism by which the Bacteria combat antibiotics, specifically β lactam based antibiotics— penicillin variants—is by producing proteins that create a β lactamase enzyme that digests the β lactam antibiotic. There is a dearth of genomic information on *Elizabethkingea spp.* that blocks our knowledge of the bacteria's other methods of pathogenesis. Scientists know three genes with correlating resistance mechanisms in all three species of *Elizabethkingea* to the following antibiotics: chloramphenicol, oxacillin, and streptomycin. The objective of this study is to sequence the DNA of *Elizabethkingea anopheles* and *Elizabethkingia miricola,* and test certain gene sequences for antibiotic resistant mechanisms, specifically Beta Lactamase proteins, while using a known sequence in *Elizabethkingea meningoseptica* as the control.

MATERIALS AND METHODS

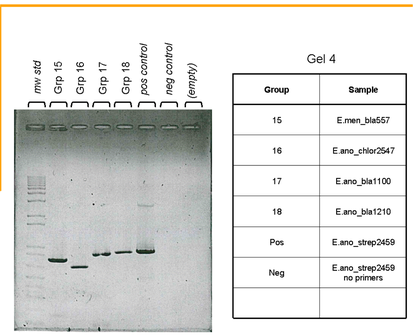
- Make a forward and backward primer for the Metallo-β-Lactamase BlaB in *Elizabethkingia meningoseptica*
- Amplify the gene with PCR amplification
- We used dH2O, Taq buffer, dNTP's (all 4), *E. meningoseptica* DNA, F-
- Check PCR Results by using Agarose Gel Electrophoresis
- Perform DNA Ligation of PCR product to the plasmid vector
- Perform the transformation of *E. coli* by heat shock
- Check for transformations by analyzing kanamycin agar plates
- Check for clones by examining plasmids and Agarose Gel Electrophoresis
- Identify if the gene is a Beta lactamase by using Nitrocefin Saturated Disks, primer, R-primer, and Taq polymerase

RESULTS

We obtained the following primers from our DNA sequence: Forward Primer: 5' ATGATGAAGAAAATGAAATGG 3' Reverse Primer: 5' TTAATTTGAAGCCTTTTG 3'



After PCR amplification we tested our genes via gel electrophoresis. The results of the gel electrophoresis showed that our gene contained 750 base pairs. This matched the gene sequence we were initially given.



Gel 4

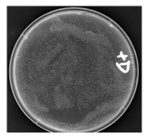
Group	Sample
15	E.men_bla557
16	E.ano_chlor2547
17	E.ano_bla1100
18	E.ano_bla1210
Pos	E.ano_strep2459
Neg	E.ano_strep2459 no primers

Transformation

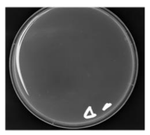
After DNA ligation of PCR we performed transformation of E. Coli by heat shock, and the resultant E. Coli were grown on kanamycin agar plates

Controls:

Positive Control: Uncut, circular Cloning vector (pSKB3)

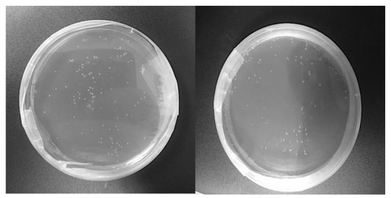


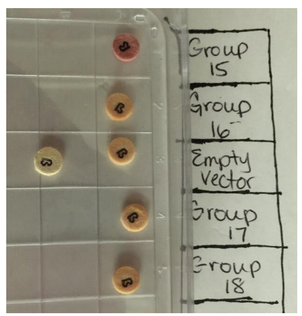
Negative Control: cut, linear Cloning vector (pSKB3)



Transformation of Metallo-β-Lactamase BlaB in E. coli

Plate 1: 130 colonies Plate 2: 120 colonies

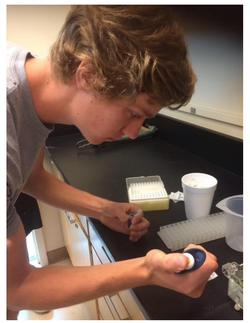


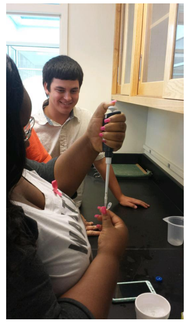


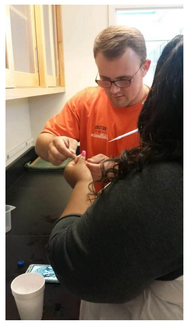
After identifying clones of the transformed E. coli, we confirmed that the genes were Beta Lactamases through a Nitrocefin Qualitative Colorimetric assay for beta-lactamase activity. Our gene, Group 15, was confirmed as a beta lactamase by turning red,. This was expected as our gene was the control, and previously confirmed in a different experiment performed by Gonzalez and Vila (2012). We did not experience any technical errors and the experiment went as predicted.

DISCUSSION

The purpose of this experiment was to test if the gene was a β lactamase gene. Our gene served as the positive control. We knew, prior to performing the experiment, that our gene was a β lactamase gene. The purpose of our portion of the experiment was to make sure the methods of the experiment weren't flawed. We followed the same instructions as the other groups to serve as a reference point for them. Our experiment was important because without our control group there is no way to know if the test was performed properly, which exposes all the results to scrutiny. The purpose of the positive control is to be ran parallel to the other experiments to test the effectiveness of the test and rule out any possible inconsistencies. Because our control showed up positive we proved that the other results creditworthy. The fact that the results were favorable proves that the test worked and eliminates further scrutiny. It means that the results of the other experiments that showed up positive were indeed a beta-lactamase. Our experiment on our β lactamase gen did not experience any inconsistencies. All the data matched that which was predicted from the DNA sequence







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