Antibiotic Resistance of *Elizabethkinga miricola*

GRP#4
Section#001

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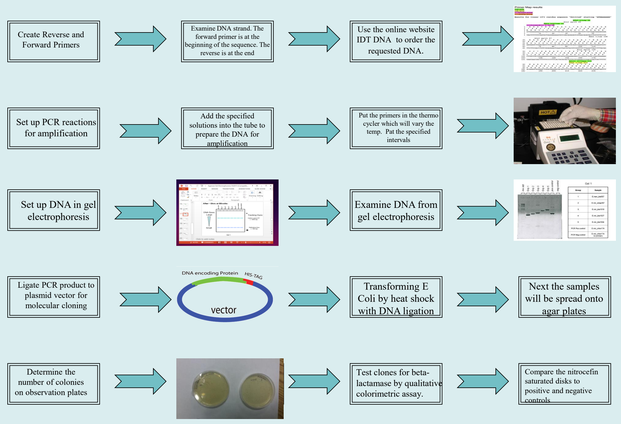
ABSTRACT

"Elizabethkingia, a bacterial species causing human disease, was found to be resistant to many different antibiotics through beta-lactamase. Antibiotics are a type of medicine used to treat bacterial infections while beta-lactamase is an enzyme that provides antibiotic resistance by breaking downs the antibiotics' structure. There are many different types of Elizabethkingia species such as *Elizabethkingia meningoseptica, miricola, and anopheles. Elizabethkingia meningoseptica* is found in nature all around us while *Elizabethkingia miricola was* found on the Mir space station. *Elizabethkingia anophelis* is found in the gut of mosquitoes and has already been tested to be resistant to more than twenty different antibiotics. In this article, we determined whether or not our given genomic sequence of *Elizabethkingia miricola* would be positive for encoding a beta lactamase gene. After PCR Amplification, DNA ligation, and heat shock transformation of E. Coli, we were able to determine whether or not our genetic sequence was a beta-lactamase gene. All beta-lactamase enzymes break the bond of the beta-lactam ring in penicillin, a strong antibiotic effective against many different bacterial infections, inactivating it making that specific bacteria resistant to that antibiotic. Nitrocefin is a chemical compound sensitive to hydrolysis of all known beta-lactamases produced by bacteria. We placed our plate transformation mixtures onto saturated nitrocefin disks to finally determine whether our mixture was positive for beta-lactamase genes. Our mixtures were positive for beta-lactamase if the disks turned red, and negative if they stayed pale yellow."

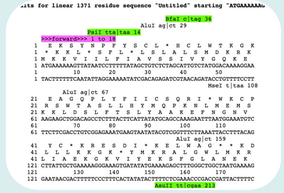
INTRODUCTION

This experiment will involve an *Elizabethkingia* species called *Elizabethkingia miricola*. *E. miricola* is a species that was initially isolated from condensation water of the space station Mir (7). *E. miricola* is resistance to multiple antibiotics. The *Elizabethkingia species* is said to have several different beta-lactamase genes. The species expresses multiple antimicrobial resistance phenotypes and is resistant to the action of several antimicrobials (1-3). Additionally, *Elizabethkingia* draft genomes have revealed that each species harbors numerous putative beta-lactamase genes (1,4,5). Beta-lactamases are enzymes produced by certain bacteria that provide resistance to β-lactam antibiotics (6). Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure. Antibiotics are a type of medicine used to treat bacterial infections; however, some bacteria have built up resistances against our medicines. The mechanisms of antibiotic resistance vary from agent to agent, but they typically involve one or more of: target alteration of the drug in the bacterial cell, enzymatic modification or destruction of the drug itself, or limitation of drug accumulation as a result of drug exclusion or active drug efflux (8). We used PCR and molecular cloning techniques to determine if a gene from *E. miricola* was a putative beta-lactamase gene or not. We designed primers that were used to drive the PCR Amplification of a region by producing products that overlap each other. After that we will use ligation to connect the DNA encoding protein to the vector. Through this experiment we hope to determine the beta-lactamase genes contained in our *E. miricola*.

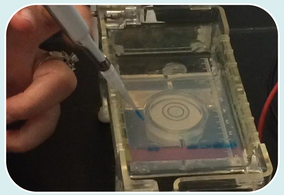
MATERIALS AND METHODS



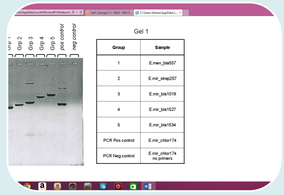
RESULTS



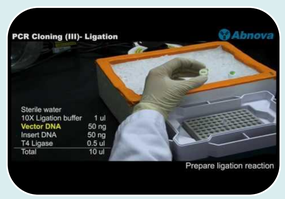
Based on the DNA sequence that was given to our group we created a forward and reverse primer along with a final reverse compliment. For our next procedure we used Taq Polymerase to template DNA, forward and reverse primers flaking the region of interest, dNTP's or DNA nucleotide building blocks to make the PCR products and a buffer with magnesium and the correct pH for optimum activity. We concluded with a total volume of 106 uL of combined reagents in our PCR tube.



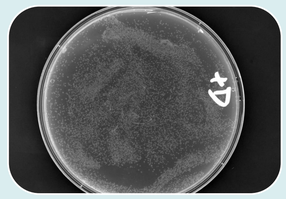
In the next procedure, we took copies of the target DNA and created an agarose gel electrophoresis in order to view and examine the DNA and predict the amount of bases in the DNA sequence. We predicted that the size of the DNA would be1.371 or 1.4 kilobases based on the amount of bases in our DNA sequence.



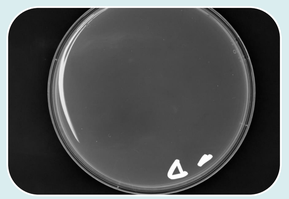
We performed the ligation of our PCR product to plasmid vector in preparation for molecular cloning. We obtained copies of our DNA and the size of our PCR product sample, which was about 1.5 kilobases.



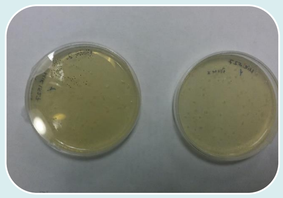
With the transformation of E. coli by heat shock with DNA ligation reactions, we were able to use the plate transformations mixtures that were spread onto Kanamycin (a broad-spectrum antibiotic) to determine gene resistance.



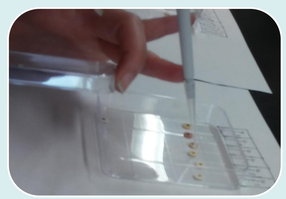
The growth of the E. coli on the petri dish shows which genes are resistant. (Positive control)



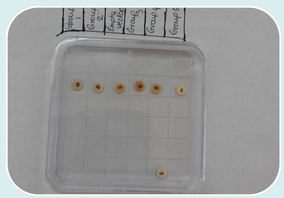
This petri dish shows an example of very little resistant gene growth of E-coli.(Negative control)



On our petri dishes, there were 200 colonies of resistant genes viewed on petri dish 1 and 232 colonies on petri dish 2.



To test if our gene was able to cleave a bond in a beta-lactamase enzyme, the teacher assistants took 20ul of the bacteria culture and placed it on the nitrocefin saturated disks.



Our genetic sequence was positive for the beta-lactamase gene but because the chemical compound nitrocifin was similar to our gene, the nitrocifin pad showed that our primer sequence was slightly resistant to a certain type of beta-lactam gene. To improve our results we could change the chemical compound used in determining if our sequence was resistant.

DISCUSSION

There has been an interest in understanding the antibiotic resistance of the Elizabethkingia bacterium. Elizabethkingia species express a multiple antimicrobial resistance phenotype and are resistant to the action of many antimicrobials (Lin et al. 2012) Additionally, Elizabethkingia draft genomes have revealed that each species harbors numerous putative β-lactamase genes (Lao et al. 2015). Elizabethkingia anophelis is a dominant species resident in the mosquito gut and also a human pathogen (Kukutla et al. 2013). When given the DNA sequence for elizabethkingia miricola we predicted that our genetic sequence would be positive for encoding a beta-lactamase gene. In another experiment, multiple antibiotic resistance-associated coding sequences were detected, including sequences encoding 26 putative β-lactamases (Quick et al 2014). Beta-lactamase provides antibiotic resistance by breaking down the antibiotics' molecular structure within the bacterium (Neu 1969). Our genetic sequence was positive for the beta-lactamase gene but because the chemical compound nitrocifin was similar to our gene, the nitrocifin pad showed that our primer sequence was slightly resistant to a certain type of beta-lactam gene. Most of the cases due to improper antibiotic use cause serious life-threatening infections (Jean et al. 2014). To improve our results we could change the chemical compound used in determining if our sequence was resistant to another antibiotic.

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