Elizabethkingia and the PCR Product

Grp 17
1990.002

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ABSTRACT

The purpose of this project was to figure out which bacteria are resistant to antibiotics, which are a type of medicine which are used to treat bacterial infections. For every single human cell, there is a bacterial cell present. The mechanisms of antibiotic resistance are to pump the antibiotic out of the cell, destroy the antibiotic, modify the antibiotic, and modify the antibiotic and to "dodge" the antibiotic. The specific species we were working with was the Elizabethkingia Anophelis, which is found in the gut of mosquitos and causes human disease. There were several steps in the process of this project and they were; Sequence the DNA, Extracting DNA from samples of interest, Sequence the Genomic DNA and determine what genes exist, PCR amplification, PCR fragment for cloning, put into E. Coli, Design primers, ligation reactions, heat shock transformation, and finally screen for Beta-Lactamase activity.

INTRODUCTION

Elizabethkingia anophelis is a strain of a possible beta-lactam bacteria that is found in the stomach of mosquito's that causes human disease. We were conducting this experiment to see if the bacteria would react to the Beta-lactam antibiotics that were administrated at the end of the experiment. While learning about *elizabethkingia*, and how to perform the experiments, we learned more about PCR and how it's applications can help research. This lead us to have a deeper understanding as to what we were doing, whether it was ligation or agarose gel application. We had the help of our professor Dr. Canaan, Shannell Shoop, Nathanial Torres, and Dr. Jessica Matts. Whenever we had questions they were there to help.

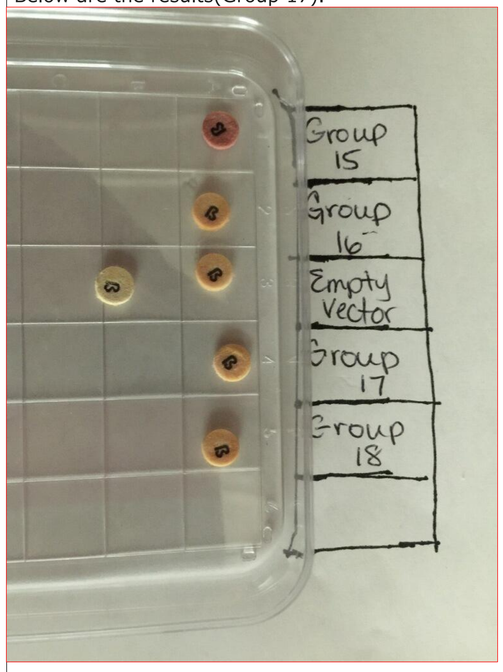
MATERIALS AND METHODS

Agarose Gel
Micro Pipette
Agar Plate
DNA
Heat Shock

We started out the experiment by creating primers for our PCR products. We didn't use any website for this, we just used the instructions from the PowerPoint and checked with each other before the primers were turned in. The forward primer was: ATGAATGCTCAGGTAGTAAAAG and the reverse primer was: TTATTTATCTTGGGAATC. After setting up the PCR reactions, we used agarose gel to see if our PCR reactions worked which they did. We then set up the ligation by using the micro pipette so we could put put the PCR product through heatshock with E. Coli cells. When we checked for cultures, we found that we had 123 cultures on our agar plate. To end it all off, with the assistance of Dr. Matts we identified true recombatiant clones and tested for beta lactamase activity.

RESULTS

To determine whether the genes we tested were true beta lacatmase genes, we used a nitrocefin tablet. Nitrocefin is a chromogenic cephalosporin substrate routinely used to detect the presence of beta-lactamse enzymes produced by microbes. In our test, the original pale yellow changed into a pale orange color, confirming that the gene assists the bacteria in resisting antibiotics. Below are the results(Group 17).



DISCUSSION

The shift in the nitrocefin tablet confirms that the gene codes for a beta-lactam. This means that the gene helps the bacteria to resist against many common types of antibiotics such as penicillin.These findings are very important because it allows us to better understand Elizabethkingia and how it resists antibiotics. The genetic information we have tested can be used to help develop more effective medicines and allows researchers to further test for other prospective beta-lactam genes by looking for other genes with similar base pairs.

REFERENCES

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