The Naturally Occurring Anomaly of Antibacterial Resistance in Elizabethkingia Anophelis

GRP# 16
Section#

Samantha Howe, Ashley Leuellen, Conner McDaniel, Adam Hernandez, Dr. Patricia Canaan, Dr. Jessica Matts, Shannel Shoop, and Nathaniel Torres.

ABSTRACT

The goal of the experiments performed over this past semester was to determine the antibacterial resistance present in Elizabethkingia Anophelis. This experiment was conducted over the majority of the semester, having the groups meet in class once a week and perform each leg of the experiment. DNA was created using the process of PCR and cloning a particular piece of the DNA sequence and testing the antibacterial resistance of the bacteria and testing weather or not there is a correlation between Beta Lactam Genes and the resistance of Elizabethkingia Anophelis.

INTRODUCTION

The bacteria known as Elizabethkingia has three separate strains that occur in various natural locations that have been revealed over time. The three strains are Meningoseptica, Anophelis and Miricola. The research performed over this semester focused on the Anophelis strain of the bacteria, this strain is spread from the bite of mosquitos and is known as showing a unique resistance to a wide variety of antibiotics (pubmed.com 2015). The interest in this topic was presented by Doctor Patricia Cannan with the specific question of weather or not their was a particular resistance to Beta Lactamase genes that are present in certain antibiotics. The experiments performed by the Oklahoma State University Biochemistry and Molecular Biology division tested various antibiotics to determine whether or not Elizabethkingia Anophelis showed specific resistance to Beta Lactam genes present within certain antibiotics to draw a definitive conclusion on the resistance of this bacterial strain.

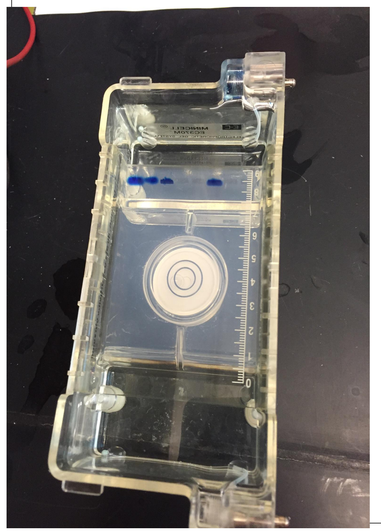
MATERIALS AND METHODS

The beginning of our experiment involved identifying the start and stop codons of chloramphenicol acetyltransferase and analyzing the original sequence of the sample given to our group. Using the reverse compliment of the start and stop sequences of the sample and plugged in the bioinfometrics.com to check if the forward and reverse primers we derived would actually work for our experiment. After confirming the validity of both primers, both were ordered online. After receiving the primers, we mixed a variety of ingredients inside PCR tubes and created the DNA and mixed in both or forward and reverse primers in order to begin the PCR process. As we mixed in certain materials and ingredients into the primary tube we made sure to keep every tube in a cup of ice to maintain a solid temperature. After all materials were added into the PCR tubes, each groups sample underwent a variety of temperature changes to denature the proteins within each tube and being the process of PCR. The next step was to utilize the process of agorose gel electrophoresis in order to see the PCR had been successfully set up. The process to ensure the PCR had worked when observing the agorose gel was to compare the specific result of our groups DNA and the fragment it created vs the predicted fragment size. The next step was setting up the DNA ligation of our PCR product by using a micropipetter and adding ingredients into the PCR tube and storing all ingredients aned tubes at the correct temperature. Heat shock transformation of the ligation mixtures. The process of heat shock transformation involved having viable E. Coli cells and mixing it with a small amount of our ligation mixture. The mixture was then held on ice for 15-45 minutes, then placed into a hot water bath for 30 seconds and then added back into the ice for three minutes to finally be placed into a room temperature "recovery broth". After adding this previous mixture to transformation plates and we counted the number of colonies present within it. A clone was then made and tested for Beta Lactam presence by placing a small sample onto a nitrocefic soaked disk.

RESULTS

Since we knew from the beginning that our results would turn out negative since we had the negative control but were able to draw data from the processes that we undertook in the beginning of the experiments.

The results on the right show our groups sample (group 16) as compared to the other groups when our sample was placed onto the nitrocefin soaked disk. A color change indicates the presence of a true Beta Lactam gene in the groups sample. We knew that we had the negative control and were expecting to see the disk stay the same color as it did at the beginning, that being a yellowish color. However our disk had a very slight color change. This was attributed to the daily condition of the "broth" that our sample was added into in order to give a substation sample.



The images on the right show some of the processes involved in the experiment that our class participated in throughout the year. As said before hand, our group was aware from the beginning of the experiment that our sample given to us was the negative control so we knew that our sample should test negative so our results in turn needed to be 100% negative in order to be an accurate control. The image on the top shows the conditions in which our samples were held in and the environment that our experiments took place. The photo of the bottom shows the process of gel electrophoresis. The results of all the experiments conducted within our group were able to prove that our sample was in fact negative. The slight color change in the stage involving the nitrocefin disks raised a question of whether or not we had made an error at some point within our experiment but we were able to attribute the change to other factors in the experiment. We are able to say that the result derived from our work that we indeed produced an accurate negative control to compare to the other groups.

DISCUSSION

For our group, we were aware from the beginning that the sample we were testing was the negative control of the experiment. The importance of this is that our results must be conclusively negative the same way the positive control needed to be positive to ensure that it could be an accurate control in reference to the other groups. Had our result been positive or the positive control had been negative, mistakes would have been made in the lab that skewed the results and this could compromise the rest of the experiment for all groups involved.

Our control did come up negative, but in the very last stage of the experiment when our sample was added too the nitrocefin soaked disk their was a very slight color change. The disks are naturally a yellow color and when a Beta Lactam is present within the added sample it will change to an orange/red color. This slight change can be seen in the final picture in our results section. Since we knew that our control was negative we had been assuming that their would be absolutely no color change. With their being a slight change their was a slight worry that we had made an error in our experiment that produced a positive result somehow. However after further analysis and looking closely at the disk, the color change was so slight that we could attribute the small change to the broth that was present in our sample at the end of the experiment that caused the minute change. However this does slightly skew the results still as the whole purpose of the negative control is for it to be definitively negative and show absolutely no color change to be a solid frame of reference to the other groups in the class for them to make a comparison against.

Our results thankfully did come out negative and were able to be a viable source as a reference. The processes we underwent throughout these past couple of weeks taught us the processes involved in PCR and while we could not necessarily directly contribute to the question of antibacterial resistance in Elizabethkingia but were able to contribute to the experiment as a whole and aid the rest of the groups and allow for the completion of the experiment as a whole and learn a new lesson in antibacterial resistance.

REFERENCES

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