Testing For Antibiotic Resistance in *Elizabethkingia miricola*

GRP#12
Section#1

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ABSTRACT

We were confronted with the problem of determining whether or not a specific gene from the bacteria *Elizabethkingia miricola* conferred antibiotic resistance. In particular, we wanted to find out if the selected gene was a beta lactamase gene. Beta lactams are resistant to penicillin, and the information acquired in this experiment could prove to have real world applications. We started with a gene sequence, and from there constructed forward and reverse primers. Then using PCR amplification, we replicated our gene billions of times. We continued by putting our amplified product through a process known as agarose gel electrophoresis. We then proceeded to ligate our PCR product with a plasmid vector in preparation for molecular cloning. Our DNA ligation was taken and added to a sample of *E. coli* via heat shock. We needed just one colony to form successfully to continue with the project, and were pleased to find that we had a total of 230 colonies. The last step was adding kanamycin broth to our colonies and observing color change. After months of long and hard work, our research found that *Elizabethkingia miricola* does in fact confer resistance to beta lactam antibiotics.

INTRODUCTION

Elizabethkingia miricola, previously known as *Chryseobacterium meningosepticum*, was used to confer antibiotic resistance. *Meningoseptica* was originally described as *Flavobacterium meningosepticum* in 1959 by American bacteriologist Elizabeth O. King. This organism is usually resistant to most antibiotics prescribed for treating bacterial infections, including extended-spectrum beta-lactam agents and aminoglycosides, a serious challenge to the patient and the treating clinicians. Beta lactams are resistant to many medications such as penicillin that have a beta-lactam ring in their structure. In doing so the beta-lactamase enzyme inactivates the antibiotic and becomes resistant to that antibiotic. Using PCR amplification we replicated our DNA and ligated a sample of the *E. coli* with heat shock. Once we counted our colonies from our results we continued on to add kanamycin broth to our colonies, once we had our clones we then went on to test for beta lactamase activity.

MATERIALS AND METHODS

- Construct primers for start and stop sequence by taking the first 22 bases of our gene and then taking the last 22 bases, finding their compliments, and then reversing their order. Then we ordered our primers.
- Using a micropipeter (with a new tip each time) we obtained 10 microliters of Taq buffer, 10 microliters of dNTP's, 5 microliters of E. miricola or E. anopheles gDNA, 2 mircoliters of our Forward-primer, 2 mircoliters of our Reverse- Primer, and 1 microliter of Taq polymerase and added it to the already measured amount (70 microliters) of dH2O in the orange labeled tube. Now PCR can be performed.
- We dyed our PCR with blue dye and put them in the wells so that electrophoresis can be performed. The copied DNA will move through the wells by size.
- Using a P20 micropipeter and disposable tips we added 2 microliters Ligase buffer, 2 microliters Linear Plasmid Vector, 4 microliters of our PCR product, and 1 microliter of T4 DNA Ligase Enzyme to the already measured out 11 microliters of dH20 in an eppendorf tube. The each ligation reaction is incubated at 16 degrees C overnight then stored in the refrigerator (4 degrees C).
- We obtained 20 microtiters of competent E. coli cells, added 2 microliters of our ligation mixture, mixed them together and it on ice for about 15 minutes, then heat shock was performed at 42 degrees Celsius for 30 seconds and returned to the ice for 2 minutes, then we added 200 microliters of a recovery both to the mixture, then the TA's incubated the mixtures at 37 degrees for 1 hour and plated the aliquots on kanamycin agar plates then incubated them again at 37 degrees overnight then put parafilm on them and transferred them to the refrigerator for storage.
- We then looked at the plates to see if we had any colonies and then counted them and tallied the total number.
- We then added 20 microliters kanamycin broth to our colonies to see if cloning was successful. If they turned red they are clones if they stayed pale yellow it was not.

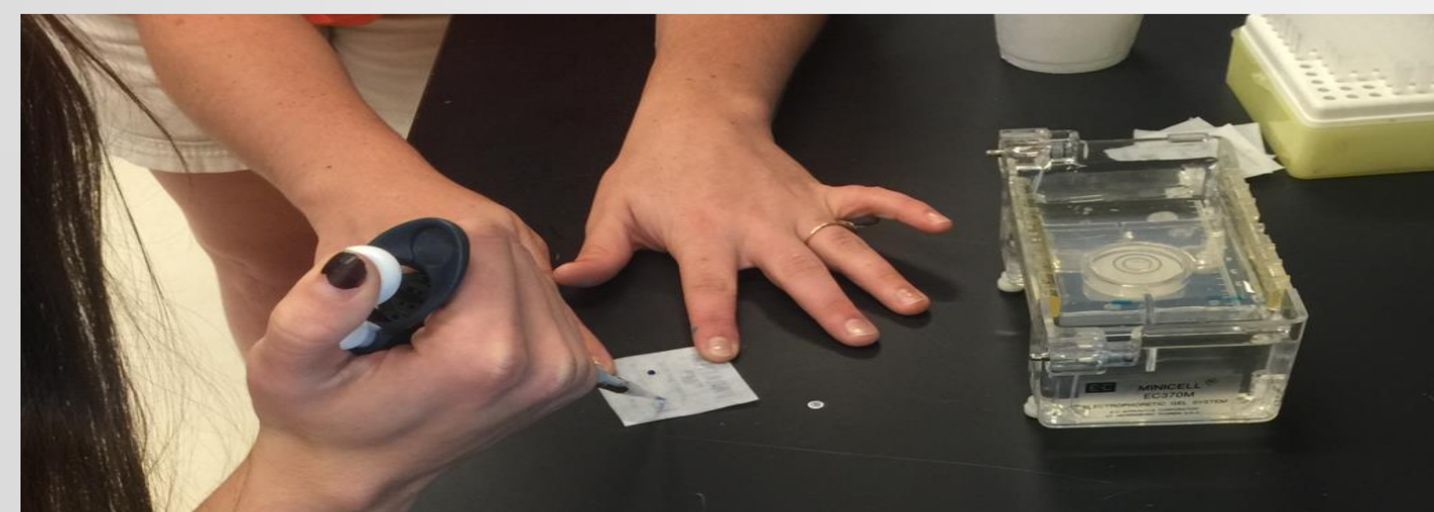
RESULTS

We found that the gene E.mir_ATCC33958_bla3617 did confer antibiotic resistance to beta-lactam antibiotics. This proves the presence of beta-lactamase production in our gene. Our initial finding in the entire experiment was that the start codon for our gene was ATGGATACCGCAAAAGTAACAGG, and the stop codon for our gene was TTATTTAGCTCTATTATTTAGC (2). Then, we prepared our Polymerase Chain Reaction in order to amplify the gene, after which we found through agerose gel electrophoresis that our PCR reaction was successful. We then performed our ligation reactions placing our targeted gene into E. coli cells, which were successful, after which we performed heat shock transformation in the E. coli cells, which produced approximately 230 colonies on our ager plate.

One anomaly to point out that we found during our research is that our kanamycin plate had a relatively small amount of colonies compared to what was expected; there were approximately 230 colonies, though there was expected to be over 1000. This suggests that there may have been an error in our methods up to that point, though the number of colonies we obtained was enough to perform our tests for antibiotic resistance with the nitrocefin disks.

Above From Left to Right: Negative Control Transformation Agar Plate; Experimental Transformation Agar Plate; Positive Control Agar Plate.
Below From Left to Right: Negative Control Nitrocefin Disk; Experimental Nitrocefin Disk; Positive Control Nitrocefin Disk.

Below: TA's assist team members in implementing Agarose Gel electrophoresis



DISCUSSION

(Beta-lactam antibiotics include penicillin, cephalosporin, and carbapenem, some of the more common antibiotics in use in the medical field today (1). Due to their common use, their effectiveness is waning, due to the tendency of natural selection to select for the bacteria which experience mutations that allow them to produce beta-lactamase, a group of enzymes which cleave the beta-lactam ring, rendering the antibiotics useless (1). This growing prevalence of bacteria which have genes that encode for the production of beta-lactamase has become a common problem in primary care medicine. This leads researchers, such as ourselves, to try to locate the genes in the bacteria *Elizabeth miricola* which confer the production of beta-lactamase, and in turn, resistance to beta-lactam antibiotics.
Our discovery that the gene E.mir_ATCC33958_bla3617 does encode for the production of beta-lactamase and antibiotic resistance is an important one in the effort for eliminating the antibiotic resistance. The genes which confer antibiotic resistance obviously must be identified before any further research can be conducted in order to eliminate them. It may be necessary in the future to locate all of the genes in *E. miricola* that encode for antibiotic resistance in order to design a solution to the issue. Also, it is important to establish that through our method of experimentation, the only thing that the change in color of our nitrocefin disk could mean is that the gene confers antibiotic resistance, as we PCR amplified the exact gene, E.mir_ATCC33958_bla3617.

REFERENCES

1. Green, O'Neil, Patrick Murray, and Juan C. Gea-Banacloche. "Sepsis Caused by *Elizabethkingia Miricola* Successfully Treated with Tigecycline and Levofloxacin." *Diagnostic microbiology and infectious disease* 62.4 (2008): 430–432. *PMC.* Web. 8 Nov. 2015.
2. Stothard P (2000) The Sequence Manipulation Suite: JavaScript programs for analyzing and formatting protein and DNA sequences. Biotechniques 28:1102-1104.