GRP10
Section 1

Searching for a bata-lactamase gene in Elizabethkingia miricola 2882: E.mir_ATCC33958_bla2882

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INTRODUCTION

Antibiotics are defined as a type of medicine used to treat bacterial infections (1). At one point, antibiotics were a game changer in the field of medicine. However, there are many bacteria that are becoming resistant to antibiotics. This is a major concern, especially considering how many bacterium are in the human body. Granted, most of this bacteria is beneficial to our bodily processes. For the bacteria that is not beneficial, it is imperative that antibiotics are able to treat the bacteria effectively. So, when antibiotics become resistant it provides a concerning conundrum. Bacteria become resistant by pumping the antibiotic out of the cell, destroying the antibiotic, modifying the antibiotic, or dodging the antibiotic. *Elizabethkingia* is one type of bacteria that is resistant to various antibiotics. Found in the gut of mosquitoes and in nature, *Elizabethkingia* can cause humans diseases. Since it is resistant to over twenty antibiotics, it is a prime candidate for this test. Beta-lactamase is secreted to digest antibiotics. *Elizabethkingia* have several different beta-lactamase genes, and therefore produce several different beta-lactamase. This allows *Elizabethkingia* to become resistant to various antibiotics.

The genome this group was testing is as follows:

>E.mir_ATCC33958_bla2882|putative MBL
ATGCTGCATATACAGATTTTTCCTTTTAATCCTTTTTCTGAGAATACTTACATCATATACAATGAT
CAGAAACAAGCATGGATTATAGATCCGGGAAATGTTCATCCAAAAGAAACGGAAGCCCTGCA
ATCTTTTATTGCCGATAATGGATTGAAAGTTGAAAAGATATTACTAACCCACGCCCACATTGATC
ACATTCTGGGACTACAGTGGGCACACGACACTTATAAAGTTCCTGTATATCTCCATCCGGATGA
AGAAGAAATTCTGAAAATGGGTAATCTTTCCGCACAACGTTTTGGATTCGATTTCGAGAATTTT
AATGGGGATCTAAAATTCCTTAATGAAGGTGATGAATTAAAATTAGGTGATGAAGTATTTCATAT
TTATTTCACTCCGGGACATTCACCAGGAAGCATCAGCTATCATAATCCGAACGGCAAGTTCATT
GTTTCCGGAGACGTTTTATTTGAAGGAAGTATTGGACGGACAGATCTGTTCAAAGCCAACTTT
GATCAGTTGATCGATAGTATTAAAACTAAACTTCTGGTTCTTCCAGAAGACACGGAAGTTTTCT
CAGGACACGGAAATCCTACTAAAATAGGATTTGAGAAAGAACATAATCCATTTTTAAAATAA

To test this sequence, a PCR machine will be used. PCR's are used to clone genes, diagnose infections, trace contamination, to name just a few uses. To extract DNA from a sample the cells first have to be ruptured, DNA is purified using organic extractions, and is finally concentrated by precipitation. Using a PCR and sequencing DNA is useful to predict and identify traits of genes. By isolating various genes and exposing them to different antibiotics can prove useful when determining exactly which gene or genes are effecting antibiotic resistance and will be the focus of this experiment.

MATERIALS AND METHODS

1.. We started off our experiment by finding the forward and reverse primer for our gene.
   Each primer had to be between 18-30 bases and end on G/C.
   The first 23 nucleotides of the DNA sequence, ATGCTGCATATACAGATTTTTCC, is the forward primer
   And using the website http://www.bioinformatics.org/sms/rev_comp.html, we took the stop codon and found the reverse primer, TTATTTTAAAAATGGATTATGTTC.
      Then submitted our primers to Professor Canaan who ordered them.
2. The next week Professor Canaan gave us the primers so we set up the PCR reactions.
   All reagents on ice and then used a micropipeter to aspirate the different samples and slowly dispense them in the 70 ul of dH_2O.
   Added 10 ul of 10 X Taq buffer, 10 ul of 10X dNTP's (all 4), 5 ul of E. miricola gDNA (58 ng/ul), 2 ul of F-primer, 2 ul of R-primer, and 1 ul Taq polymerase.
      Turned in our final solution to Professor Canaan who ran it through a thermocycler.
3. Then we used agarose gel electrophoresis to determine if the PCR worked.
   We used a pipter extracted our Taq DNA solution and added it to the DNA gel electrophoresis, so the lab assistants could run it through the machine. Which separates by size and shows it with tracking dyes, xylene cyanol and Bromophenol blue.
4. Set up ligation reactions of our PCR product with the vector DNA
   We took the vector DNA and our PCR product and glued them together by using a pipter to add the necessary samples.
   2 ul of 10X Ligase buffer, 2 ul of Linear Plasmid Vector, 4 ul of PCR product, 1 ul of T4 DNA Ligase Enzyme to 11 ul of dH_2O.
      Turned in our final solution to Professor Canaan who stored it over night at 16 degrees Celsius.
5. Next we did heat shock transformation of ligation mixtures into E. coli cells
   For the transformation of E.coli by heat shock we obtained 20 ul of competent E. coli cells, added 2 ul of our ligation mixture, "flicked" 3x to mix and then places on ice for 15-45 minutes.
   Then the solution went through heat shock at 42°C (water bath) for 30 seconds, it then returns to the ice bath, where we added 200 ul recovery broth (at room temperature).
      We turned the samples in to our TAs, and our TAs incubate sample at 37°C for 1 hour, plated aliquots on kanamycin agar plates, incubated at 37°C overnight, and parafilm and transferred plates to the refrigerator for storage.
6. Checked transformation plates for transformants
   Counted number of colonies per plate. (See Results)
7. With the assistance of Dr. Matts, true recombinant clones were identified.
   Screened individual colonies for the cloned fragment.
8. A clone was tested, by the TA's, for beta-lactamase activity
   Using nitrocefin saturated disks, we tested for beta lactamase activity by qualitative colormetiric assay.
   We took the nitrocefin saturated disk and applied our clone, and then let it sit for 30 minutes to see if the disk changed color to red, which would prove that our clone of *Elizabethkingia miricola*. Is a bata lactamase gene.

Results

Our Nitrocefin saturated disk did not change allowing us to conclude that our gene of *Elizabethkingia Miricola* was not a bata-lactamase gene. During the experiment we had many successful results leading up to this conclusion.

After we ordered and received our PCR primers, we PCR amplified our bata-lactamase predicted gene putting our sample through thermocycler and adding it to an argrose gel. We used electrophoresis to extract the DNA and examine if the length of our DNA which matched with the number of base pairs in our gene (shown in figure 2). We then used ligation of PCR product to plasmid vector for molecular cloning followed by heat shock

- Figure 1 shows the insertion of our PCR amplified gene into the argrose gel
- Figure 2 is our extracted DNA through electrophoresis after ordering PCR primers, PCR amplification through thermocycler then placed in argrose gel
- Figure 3 This is our plate which shows the number of transformants after DNA ligation.
- Figure 4 displays our nitrocefin saturated disks which did not change color allowing us to conclude our specific gene was not a bata-lacatamase gene.

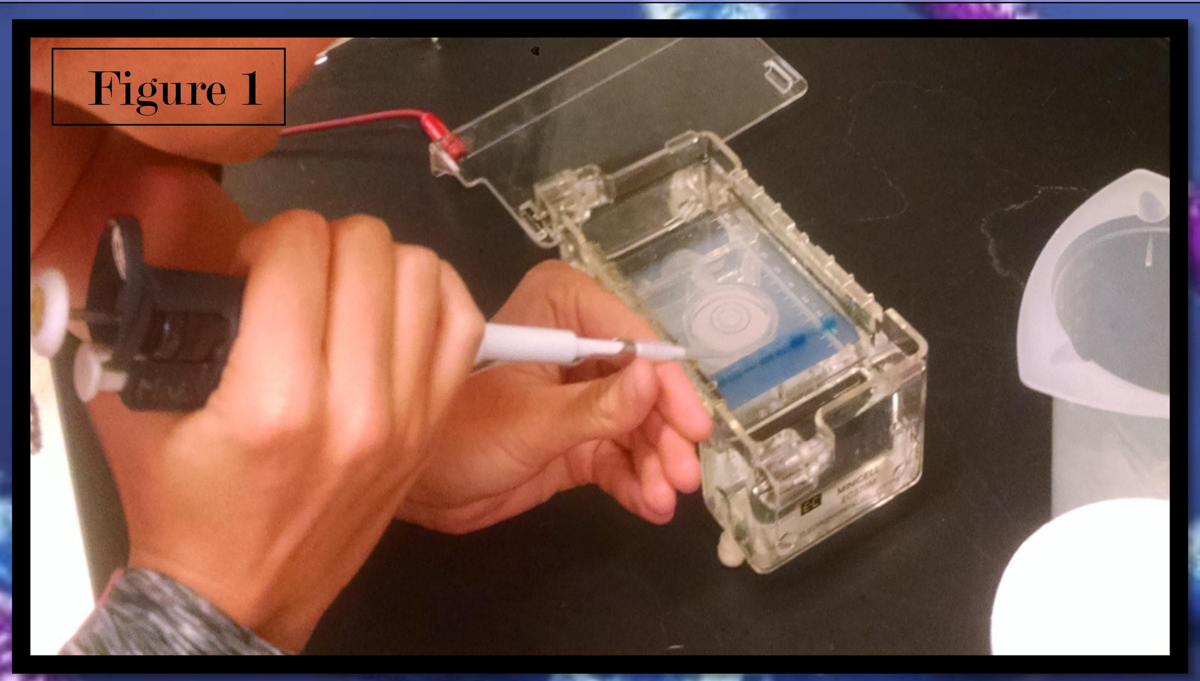
Figure 1

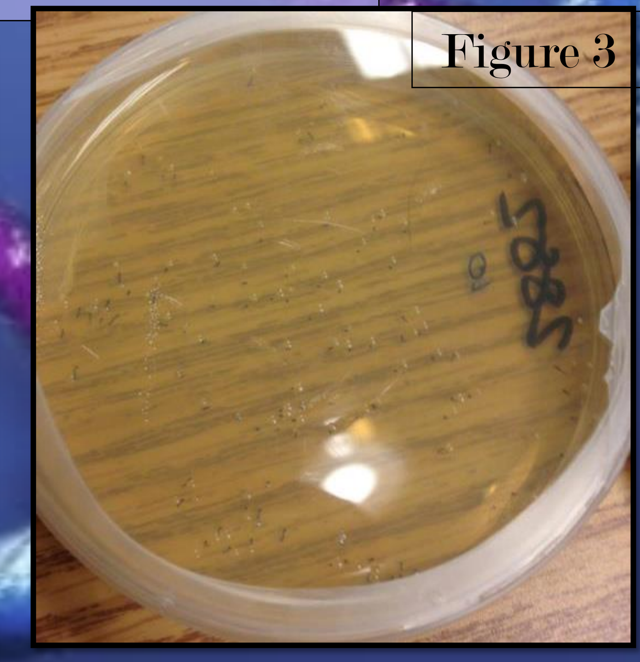
Figure 3

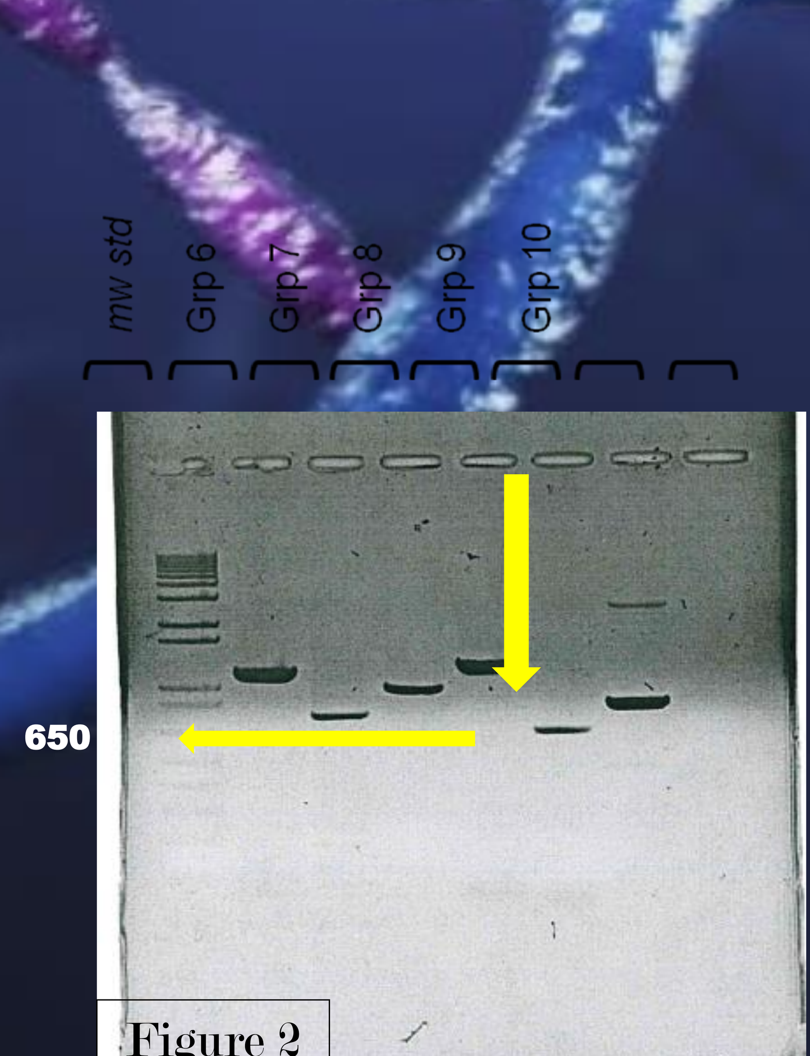

Figure 2

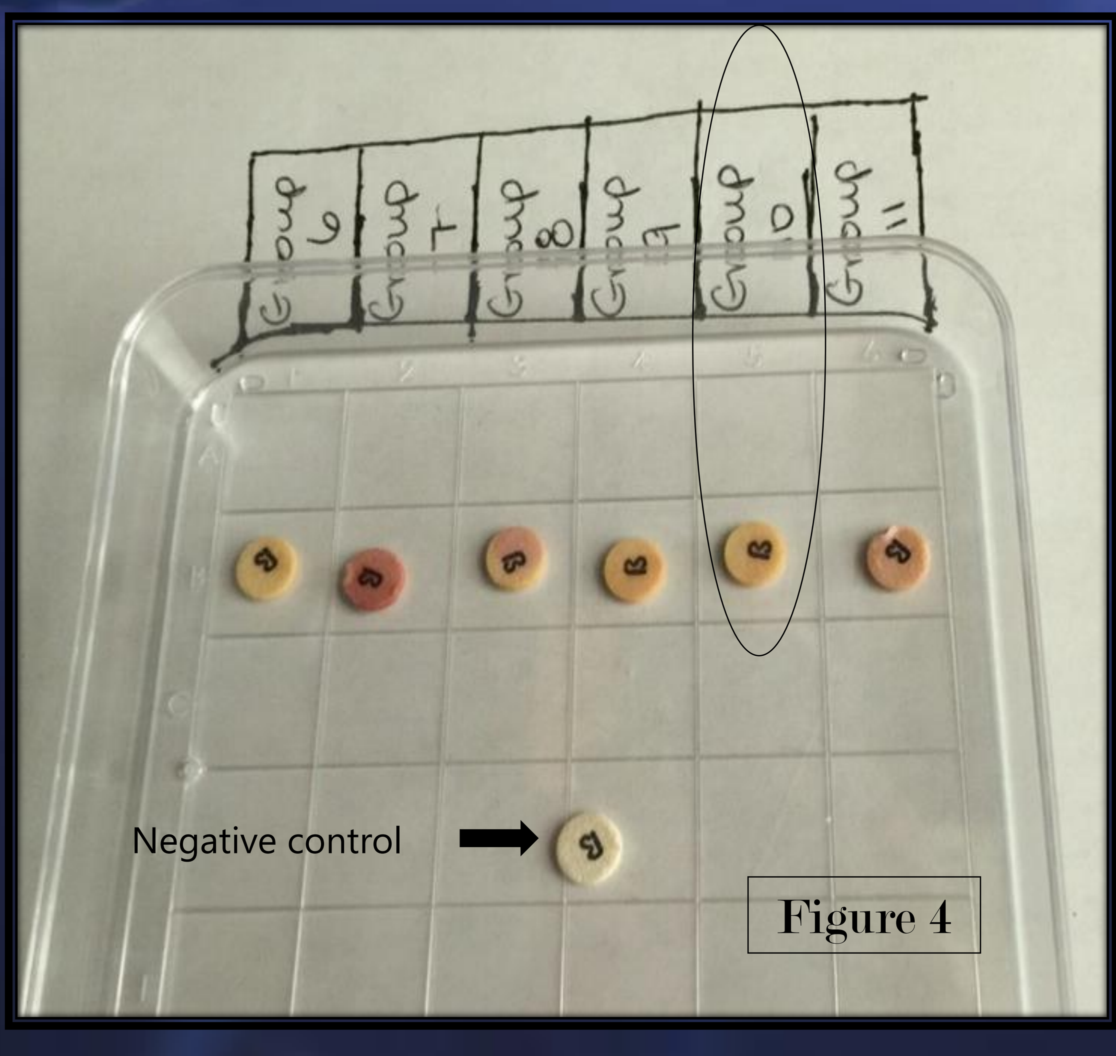

Figure 4

Abstract

Elizabethkingia miricola isolated from the Mir space station and causes human disease(2). We took a putitive antibiotic resistance gene and pcr amplified it and cloned the DNA to study it and determine if it is a bata lactamase gene. After intensive research we found our specific gene was not a bata lactamase gene.

DISCUSSION

The results of this experiment have shown that the primers were correct, which was determined by researchers who analyzed their Agarose Gel Electrophoresis results. Due to positive control producing a result, it can be concluded that the results of the Agarose Gel Electrophoresis are valid. A PCR test was ran on the primers where millions of copies of our primers were produced in order to study it. It is known that the reaction was performed correctly because the results matched up with the molecular weight standard ladder. Also, it is known that the ligase and electroshock experiments were done correctly because a very large number of colonies were formed and seen on inoculated plates. Reseachers saw that a large number of colonies were transformed, allowing a rather large size to be sampled during the nitrocefin qualitive colorimetric assay for beta-lactamase resistance. Last but not least, research can conclude that group 10's gene codes for their primer for beta-lactamase resistance was a not a bata lactamase gene due to the lack of color change on the nitrocefin disks when being tested and expected results came back with the positive and negative controls. Everything matched up well and gave us the results we needed. Without one thing coming back correct, we never would have been able to continue.

REFERENCES

1. Antibiotic. (n.d.). MedicineNet. Retrieved November 8, 2015
2. Matyi SA, Hoyt PR, Ayoubi-Canaan P, Hasan NA, Gustafson JE. 2015. Draft genome sequence of strain ATCC 33958, reported to be Elizabethkingia miricola. Genome Announc 3(4):e00828-15. doi:10.1128/genomeA.00828-15.
3. Canaan, P. (2015, August-October). Lecture presented in Noble Research Center, Stillwater, OK.