Discovering the beta-lactamase Resistance Genes of *Elizabethkingia miricola*

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GRP 3
Section 001

INTRODUCTION

In 2003, Dr. Y. Li et al. published a journal article detailing their discovery of *Elizabethkingia miricola.* In 2011, Hayek et al. recorded a case involving *Elizabethkingia meningosepticum* causing meningitis in a 68 year old woman. This leads scientists to believe that because *Elizabethkingia miricola* and *Elizabethkingia meningosepticum* are a part of the same genus, *Elizabethkingia miricola* could have antibiotic resistance similar to *Elizabethkingia meningosepticum*.

Researchers have performed an experiment testing the antibiotic resistance of the bacteria *Elizabethkingia miricola* using beta-lactamase and other reagents. Teaching assistants also helped grow the bacteria and extracting its DNA outside of class time. Other researchers have had success with a procedure which tests beta-lactamase resistance (Marchou, 1987). Similar procedures were used to test beta-lactamase resistance of a gene cloned from *Elizabethkingia miricola*.

MATERIALS AND METHODS

The experiment began with procedure necessary for cloning group 3's assigned DNA sequence. To do this, researchers identified start and end primers for the targeted gene. About 18-30 nucleotides were chosen from the beginning and end of the sequence. The complements of the start and end sequences were found to create the start and stop primers. These specific primers were submitted and ordered by Dr. Canaan and were produced by a commercial laboratory.

Next, researchers prepared the Elizabethkingia miricola DNA, which was extracted by the teaching assistants before the experiment began, for Polymerase Chain Reaction (PCR). Using a P20 micropipeter and disposable tips, researchers added the following to a test tube: 70 µL of dH_2O, 10 µL of 10X Taq buffer, 10 µL of 10X dNTPs, 5 µL of E. miricola (58 ng/L), 2 µL forward primer, 2 µL reverse primer, and 1 µL Taq polymerase. After all reagents were added, researchers used a thermocycler, to provide the proper temperature conditions for the correct amounts of time in order for PCR to occur, for 30 cycles.

Next, the PCR copied DNA was prepared for agarose gel electrophoresis. The agarose gel was prepared by other research faculty. The molecular weight ladder, positive control, and negative control were also loaded into agarose gel by faculty. Researchers added bromophenol blue dye to the copied DNA and loaded them into the agarose gel wells. The gel was then run through an electromagnetic field until the samples were about 75-80% of the way through the gel. Photographic records were taken of the gels for analysis.

After agarose gel electrophoresis, DNA samples were prepared for ligation to bacterial plasmids. Using a P20 micropipetter and tips, researchers combined the following into a test tube: 11 µL distilled water, 2 µL of 10X ligase buffer, 2 µL linear plasmid vector, 4 µL PCR product, and 1 µL T4 DNA ligase enzyme. Each tube was incubated for one night at 16 C° and then refrigerated until the next class.

Next, researchers prepared the samples for heat shock transformation. Researchers added the 2 µL of the ligation mixture to 20 µL of E. coli cells. Samples were mixed and were then allowed to process for 30 minutes. Heat shock was then performed by placing the samples in a water bath at 42 C° for 30 seconds. Samples were returned to ice for 2 minutes. Afterwards, 200 µL of recovery broth was added to the samples and was incubated. for 1 hour at 37 C° . Researchers then inoculated plates with the transformed E. coli. Plates were incubated overnight at 37 C° . Afterwards, plates were refrigerated for a week.

Next, researchers used quadrant estimation to estimate the number of colonies present on plates.

After estimation, the cells were inoculated in liquid culture. These cultures were then applied to beta-lactamase sensitive disks which change color when cells that produce beta-lactamase antibiotics are added to them.

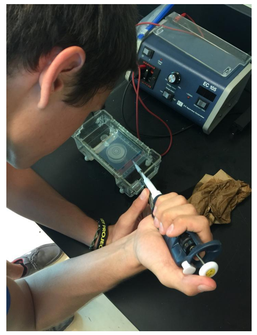
RESULTS

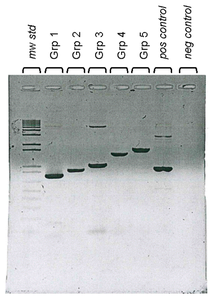
Researchers found four sets of important results in their experiments.
i.The first set of results the researchers found were the primers that were used for Polymerase Chain Reactions. The primers were

TACCGATTTTTTCGATAAAAC and CTATTTTTTAGTGTAATAATC

Start codon *Stop codon*

ii. The second set of results the researchers found were the measurements taken from Agarosre Gel Electrophoresis.

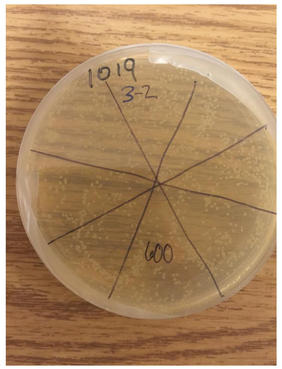




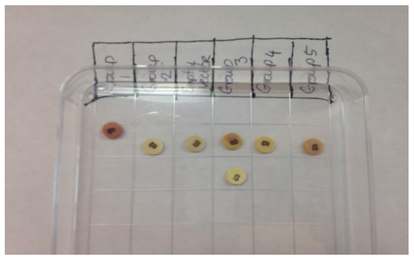
Gel 1

Group	Sample
1	E.men_bla557
2	E.mir_strep257
3	E.mir_bla1019
4	E.mir_bla1527
5	E.mir_bla1534
PCR Pos control	E.mir_chlor174
PCR Neg control	E.mir_chlor174 no primers

iii. The third set of results the researchers found were the quartile colony estimation of the transformed e. coli cultures. Researchers determined that there were 600 colonies on plate 1907-3-1 and 560 colonies on plate 1907-3-2 for a total of 1160 colonies.



iv. The fourth set of results the researchers found were the results of the Nitrocefin Qualitative Colorimetric Assay for beta-lactamase resistance.



ABSTRACT

In this experiment, researchers used polymerase chain reactions, ligation reactions, electroshock treatment, and nitrocefin qualitative colorimetric assays to determine if a targeted gene from Elizabethkingia miricola codes for beta-lactamase resistance. We found that all preliminary results proved successful experimentation. The final set of results from the nitrocefin qualitative colorimetric assay showed that the gene we targeted and used codes for beta-lactamase resistance. This is an important step in discovering the specific gene sequences that code for beta-lactamase resistance in Elizabethkingia miricola.

DISCUSSION

Researchers determined that the primers were correct when they analyzed their Agarose Gel Electrophoresis results. Because the positive control produced a result and the negative control did not produce a result, researchers can conclude that the Agarose Gel Electrophoresis results are valid. Researchers know that the Polymerase Chain Reaction was performed correctly because the Agarose Gel electrophoresis results matched properly to the molecular weight standard ladder. Furthermore, it is known that the ligase and electroshock experiments were successful because a large number of transformed colonies were observed on the inoculated plates. Researchers found a large number of colonies were transformed, allowing for a large sample size to be used during the nitrocefin qualitative colorimetric assay for beta-lactamase resistance.
Finally, researchers can conclude that this gene codes for beta-lactamase resistance because a color change was observed on the nitrocefin disks when samples were tested and the positive and negative controls had expected results.

REFERENCES

Dr. Li, Y. et al. *Emerging Microbes & Infections* (2013) **2**, e17; doi:10.1038/emi.2013.16 Published online 10 April 2013

Marchou, B., Bellido, F., Charnas, R., Lucain, C., & Pechere, J. (1987). Contribution of beta-lactamase hydrolysis and outer membrane permeability to ceftriaxone resistance in Enterobacter cloacae. *Antimicrobial Agents And Chemotherapy,* 1589-1595.