Testing for beta-lactamase genes in the bacterial strain *Elizabethkingia anophelis*

GRP 26
Section 2

Kolt Wood, Kamber LeBlanc, Kenneth Rogers, Matthew Morgan, Dr. Patricia Cannan, Dr. Jessica Matts, Shanell Shoop, and Nathaniel Torres

ABSTRACT

In our experiment, we tested antibiotic resistance in *Elizabethkingia anophelis*. We did this experiment to help form a better understanding for which antibiotics can potentially cure meningitis. Several methods, such as cloning DNA, aragose gel electrophoresis, DNA ligation reactions, and heat shot transformation enabled us to properly test the our strand for resistance. We found that our strand was resistant to the Beta-lactamase antibiotic.

INTRODUCTION

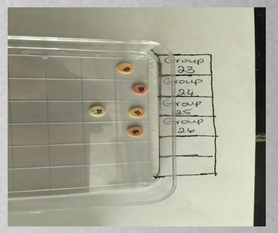
Elizabethkingia was given its name in honor of Elizabeth King who first found bacteria associated with meningitis in infants (Kim, 2005). Elizabethkingia is resistant to less than 20 antibiotics (Canaan, 2015) There are several strands of *Elizabethkingia* such as*: Elizabethkingia miricola, Elizabethkingia meningoseptica*, and *Elizabethkingia anopheles* (Kim, 2005). In our experiment, we tested *Elizabethkingia anopheles*. The disease is found inside of mosquitos, allowing it to easily be transferred to humans. By determining if our gene of *Elizabethkingia anopheles* was a beta lactamase, we were able to determine whether it was resistant to the beta lactamase antibiotic. Beta-lactamases are enzymes produced by some bacteria, which causes resistance to antibiotics such as penicillin, cephamycins, and carbapenems. Beta-lactamases break down the antibiotics' structure, causing resistance to the antibiotic (Rawat, 2010). We hypothesize that our gene of *Elizabethkingia anopheles* will be resistant to the Beta-lactamase antibiotic.

MATERIALS AND METHODS

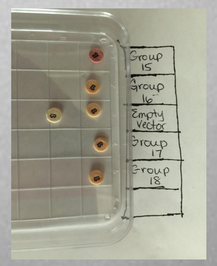
1. Designed a primer for the DNA sequence Elizabeth Kenya group 26 and confirmed it on the website www.bioinformatics.org/sms2/primer_map.html, forward and reverse primers were ATGGCTAAAAAAGCTATTTTG and TTATTTTTTAGTATAGTAATC respectively.
2. PCR was set up to amplify the desired piece of DNA in order to generate billions of copies of the specific DNA sequence through the following steps: Used a micropipette and disposable tips to aspirate and dispense reagents into a common tube for PCR amplification. The reagents are as follows;10X Taq buffer, 10X dNTP's, E. amophelis gDNA, forward primer, reverse primer, and Taq polymerase. The 100 µl PCR mix contained 290 ng of gDNA, 0.6 µM of Forward primer, 200 µM of PCR nucleotide mix (all 4 dNTPs from Fisher Scientific, Pittsburgh, PA), 1.75 mM MgCl2, and 2.5 units Taq polymerase (M1865, Promega Chemicals, Madison, WI). The PCR reaction that took place went through initial denaturation at 96°C for 30 sec, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, extension at 72°C for 1.5 min, concluding with a final extension of 10 min at 72°C, and stored at 4°C
3. Viewed and examined the copied DNA made by PCR through the following steps: DNA gel electrophoresis was used to separate the DNA sample based on size (This was performed in order to examine the copied DNA made by PCR), the smallest samples separated the fastest and the large ones separated the slowest, which therefore revealed the size of the DNA to confirm the correct DNA sequence had been amplified. *The gel used was agarose gel
4. A ligation reaction was prepared by the following steps: The amplified DNA was inserted into a bacterial plasmid vector backbone, A P20 micropipette was used to combine the following ingredients: dH2O- 11 µl, 10X Ligase buffer- 2 µl, linear Plasmid Vector- 2 µl, PCR product- 4 µl, T4 DNA Ligase Enzyme- 1 µl, the Ligation reaction was incubated at 16 degrees Celsius overnight, and samples were stored at 4 degrees Celsius until the next week
5. The recombinant DNA was inserted into E. Coli cells through a heat shock transformation through the following steps: 20 µl of competent E. coli cells obtained, 2 µl of ligation mixture added, "Flicked" 3x to mix, placed on ice for 30 minutes, heat shocked for at 42°C (water bath) 30 seconds, returned to ice for 3 minutes, added 200 µl recovery broth (at room temperature), turned samples in to Tas, incubated samples at 37°C for 1 hour, put on kanamycin agar plates, incubated at 37°C overnight, and parafilmed and transferred plates to refrigerator for storage
6. The number of vectors in the E. coli bacteria were found through the following steps: The sample spread onto the plates was analyzed, and the amount of replicated vectors were which were resistant to Kanamycin were counted.
7. Individual transformants were tested to see if they contain the DNA encoded protein through the following steps (done by graduate students): Transformants were grown in Kanamycin broth tubes and plasmids were screened for clones by Agarose Gel Electrophoresis (described in step 3)
8. The clones were screened for beta-lactamase activity through the following steps: Using Nitrocefin saturated disks and a micropipette, the target DNA sequence was inserted into the Nitrocefin saturated disks (done by graduate students) to see if it would generate a red color and confirm resistance to beta-lactam antibiotics.

RESULTS

Our group was assigned gene numbered twenty-six. We were testing this gene to see if it was a beta-lactamase. We theorized that it was due to the similarities between the sequencing of our gene to similar genes that are known beta-lactamases. To test the gene after it had went through the cloning process we used a Qualitative Colorimetric Assay for beta-lactamase activity. For the assay we used Nitrocefin saturated disks. The Assay would turn red from white when detecting the presence of a beta-lactamase gene after the solution is applied. Our sample is shown below and did in fact turn red.



Our objective in this experiment was to isolate, clone, and then test a specific gene for beta-lactam resistance, and throughout experiments we deduced that it did indeed confer resistance. This also aligned with our theory that it was a beta-lactamase. There were twenty-two other experiments similar to ours. Out of those twenty-two there were nine other genes that confers beta-lactam resisitance.



Pictured in group fifteen and sixteen are the positive and negative controls respectavly

DISCUSSION

Our results have detected a beta-lactamase.This is a mechanism used to confer resistance to a bacteria to beta-lactams, which is a type of antibiotic, (Garua). The positive control for the known beta-lactamase had coloration, as did our experimental group, confirming antibiotic resistance Having found a beta-lactamase means that we are one step closer to completely understanding the full structure of Elizabethkingia anophelis. *Elizabethkingia anophelis* is a bacteria found in the gut of a mosquito and confers a type of meningitis.This is the first step to solidifying that these nine genes are truly beta-lactamases. Now there must be further testing to truly see if these nine genes are beta-lactamases.

REFERENCES

Gianpiero Garau, Isabel García-Sáez, Carine Bebrone, Christine Anne, Paola Mercuri\, Moreno Galleni, Jean-Marie Frère and Otto Dideber1,Antimicrob. Agents Chemother. July 2004 vol. 48 no. 7 2347-2349

Kim, K., Kim, M., Lim, J., Park, H., Lee S. 2005. Transfer of Chryseobacterium meningosepticum and Chryseobacterium miricola to Elizabethkingia gen. nov. as Elizabethkingia meningoseptica comb. nov. and Elizabethkingia miricola comb. nov. International Journal of Systematic and Evolutionary Microbiology., 55, 1287-1293

Rawat, D., & Nair, D. (2010). Extended-spectrum β-lactamases in Gram Negative Bacteria. Journal of Global Infectious Diseases