Determination of Beta-lactamase Activity In a Specific Genomic Sequence From *Elizabethkingia anophelis*

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ABSTRACT

Elizabethkingia anophelis **is a strain of bacteria found in *Anophelis gambiae,* a type of mosquito. Genomic sequencing of the bacteria reveals several genes that are thought to be beta-lactamases. Beta-lactamases encode antibiotic resistance to beta-lactam drugs, making treatment of this bacteria difficult. Our experiment focuses one one of these gene sequences to determine whether or not it is a true beta-lactamase. PCR amplification is used to replicated our specific gene. The DNA was then inserted into vectors by ligation. Lastly, Nitrocefin was used to determine the beta-lactamase quality of our specific gene. Our results indicate that our DNA sequence does not encode a beta-lactamase gene.**

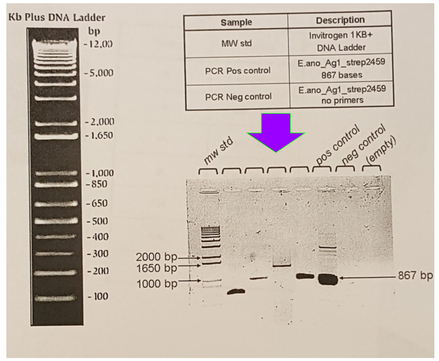
INTRODUCTION

Elizabethkingia anophelis **is one of the dominant bacteria in the gut of *Anophelis gambiae*, a type of malaria vector mosquito (Li et al. 2015). *E. anophelis* can cause acute illnesses; however, this bacteria contains genes that encode resistance to several different antibiotics, making it difficult to treat those who are sick. One of the causes for antibiotic resistance in *E. anophelis* is the beta-lactamase enzymes that resist beta-lactam antibiotics, such as penicillan (Kukutla et al. 2014). The beta-lactamase enzymes can cause enzymatic degradation of drugs, alteration of the target drug site, or the riddance of the drug through efflux pumps. Our research uses a cloning approach and Nitrocefin to test one of the genomic sequences to determine whether or not it encodes a beta-lactamase enzyme.**

MATERIALS AND METHODS

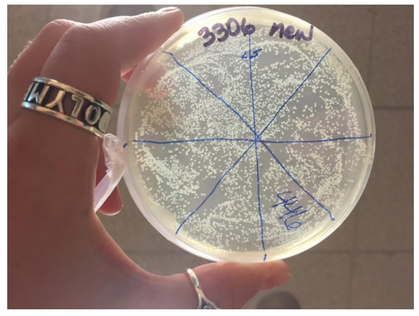
We designed primers from our putative beta- lacatmase and ordered them from IDT DNA.
Forward: ATGTACAAAACGTTATTCACTTCC, Reverse: CTATTCTTTAGTAAAGAGAATATC
Using a P20 micropipeter, we mixed the primers with the reagents (dH_2O, 10X *Taq* buffer, 10X dNTP's, *E. anophelis* gDNA, and *Taq* polymerase) needed to prepare the putative beta- lactamase for PCR. With the substance prepared, the DNA underwent 30 cycles in a thermocycler with constant changing temperatures of 95ºC, 55ºC, and 72ºC designed to replicate the wanted DNA fragment.
An Agrose gel electrophoresis was ran to determine if the PCR successful. Blue tracking dye was added to the DNA , which allowed us to determine the DNA's fragment size by comparing it to the bands present in the gel.
The PCR product was ligated into a pSKB3 plasmid, which also included a gene that encoded Kanamycin resistance.We mixed dH2O, 10X Ligase buffer, the linear plasmid vector, the PCR product, and the T4 DNA ligase enzyme into one test tube. The mixture was put into an incubator at 16 degrees overnight followed by refrigeration to force the *E. coli* into the plasmid.
Dr. Canaan then gathered the treated *E. coli* cells and we added the ligation reaction into a test tube. We put the mixture on ice for 45 minutes, inserted it into a water bath at 42ºC for 30 seconds, and finally returned it to ice for 3 minutes to heat shock the mixture. After the heat shock, we added recovery broth to the mixture and placed it in an incubator for an hour. The aliquots were placed on kanamycin agar plates and left them in an incubator overnight. The Kanamycin destroyed any *E. coli* colonies that did not receive our DNA. Nathaniel Torres took colonies from the plates and grew them in broth, and ran another agarose gel electrophoresis to look for the ones that have the insert. Nitrocefin was used on selected colonies to qualitatively determine beta-lactamase activity.

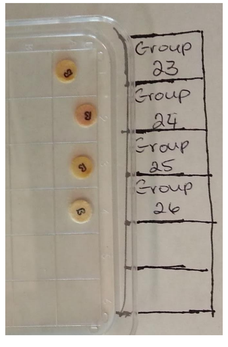
RESULTS

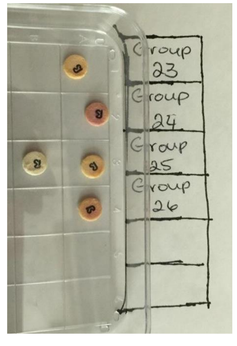


We expected the PCR amplified DNA to be 1,314 base pairs in length. The results of the agarose gel electrophoresis (pictured to the left) reveiled that the DNA was successfully replicated and ready to be inserted into the plasmid vector.

After the DNA was ligated into the plasmid vectors encoding Kanamycin resistance, they were inserted into treated *E. coli* cells. The cells were placed on a plate smeared with Kanamycin, and the colonies that contained our DNA were left on the plate. Counting of two plates (one pictured to the left) revealed 6,128 colonies total.





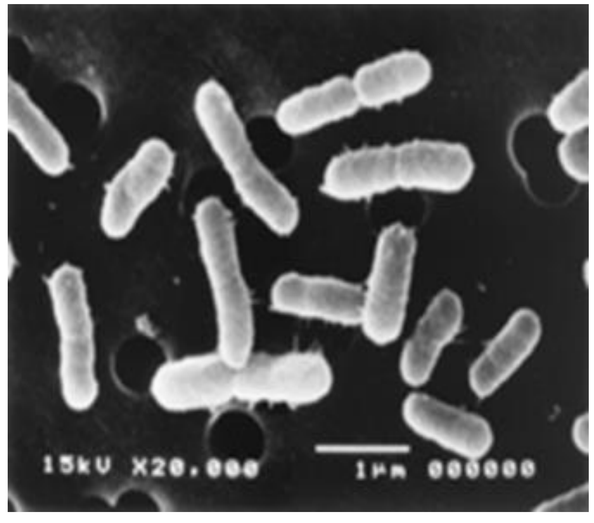


We used a qualitative colorimetric assay for beta-lactamase activity using nitrocefin. If the tablet is a pale yellow, then it was not hydrolyzed and the beta-lactamase stayed intact. If the tablet turns red, then that ensures us that the beta- lactamase hydrolyzed (opened up) the ring and is the positive result. Our putative beta- lactamase fragment kept the nitrocefin-added tablet yellow, resulting in a negative Kanamycin resistance beta- lactamase.

DISCUSSION

From our results, the gene that we were assigned and cloned into *E. coli* did not demonstrate beta-lactamase activity. Therefore, our assigned gene is not a beta-lactamase gene. However, because Nitrocefin is only a synthetic beta-lactam compound, we need confirm our negative result. One way we can prove this is to grow up our clone in the presence of a clinically used beta-lactam antibiotic. If our clone is not able to survive in the presence of the clinical beta-lactam antibiotic, this will confirm that our assigned gene is not a beta-lactamase gene.

Although, our gene did not demonstrate beta-lactamase activity, this does not mean that *E. anopheles* is susceptible to beta-lactam antibiotics. In fact, *E. anopheles* has been shown to be resistant to beta-lactam antibiotics. Therefore, there must be a beta-lactamase gene within this organism that needs to be characterized.



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- **Chuen Chew, Su, M. Givskov, F. Lauro, Y. Li, Y. Liu, M.M.S. Salido, J. Teo, L. Yang. 2015. Complete Genome Sequence and Transcriptomic Analysis of the Novel Pathogen Elizabethkingia anophelis in Response to Oxidative Stress. Oxford Journal 7(6): 1676-1677.**