Will ß-lactams Save You or Will You Meet Certain Death?

GRP #23
Section#2

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ABSTRACT

The purpose of this experiment was to determine which gene or genes in *Elizabethkingia meningoseptica* encode for the enzyme Beta-lactamase. *E. meningoseptica* was originally described as Flavobacterium Meningosepticum in 1959 by American bacteriologist Elizabeth O. King. It is ubiquitously found in water and soil, but because of its survival in hospital environments, outbreaks can occur due to exposure to a contaminated water source or medical devices (1). This bacterium /pathogen is resistant to multiple antimicrobials commonly used for bacteria and conventional empirical antimicrobials targeting those organisms may result in unfavorable outcomes (5).

At the beginning of the experiment we were given a DNA strand, E.ano_Ag1_bla3134|putative MBL, that contained 16-30 bases. After that we designed a forward and a reverse primer in order to use the PCR method. Through this method we generated thousands to millions of copies of the gene we were assigned. It was then dyed for gel electrophoresis, which indicated how many bases were in our gene sequence. Our sample corresponded with 639 bases on the molecular weight standard, it was also the amount we counted ourselves. The ligation reaction and heat shock was used to transform the plasmid into E. Coli along with gene that is known to encode for Kanamycin resistance, so as to easily test if transformation occurred. The next few steps after that were done by our professor and TA's because *E. meningoseptica* is classified as level II by the CDC and we therefore did not have the training to work with it directly. We, group 23 concluded our research with the finding that our starting DNA strand was indeed a beta-lactamase but that it had a low capacity of resistance for the antibiotic that we were testing it for.

INTRODUCTION

Elizabethkingia meningoseptica is a bacteria that is found throughout nature in food and water sources, it can also be found in hospitals because it grows "on moist surfaces such as sinks…[and] saline solution[s]" (1). This bacterium is the known cause for many infections, the most common is "meningitis in neonates with morality rates of about 57%" (1). It is also known that *E. meningoseptica* is resistant to about 20 antibiotics (class notes), some of which contain beta-lactam rings. These types of antibiotics "work by inhibiting cell wall biosynthesis in the bacterial organism" (2). Bacterium mechanisms to resist antibiotics are all the work of proteins, said proteins are encoded in the bacterium's genes (class notes). *E. meningoseptica* resistance to antibiotics containing beta-lactam rings is given by the enzyme beta-lactamase. Beta-lactamase works "through hydrolysis" by breaking open the "beta-lactam ring open, deactivating the molecule's antibacterial properties" and is "usually secreted" (4). The purpose of this experiment was to determine which gene or genes in *Elizabethkingia meningoseptica* encode for the enzyme Beta-lactamase. We hypothesis that the gene our group was given to test will encode for "the lactamase enzyme [that] breaks the beta-lactam ring open" (4).

MATERIALS AND METHODS

Materials:

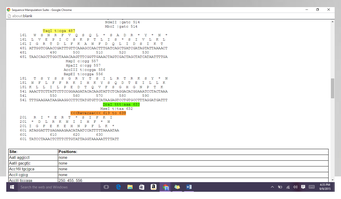
- >E.ano_Ag1_bla3134|putative MBL
- o F-primer
- o R-primer
- Micropipette
- Pipette tip covers
- dH2O
- 10X Taq buffer
- 10X dNTP's
- E. miricola or E. anophelis gDNA (58 ng/µl)
- Taq polymerase
- electrophoresis tray
- 2µL of blue dye
- Ligase buffer
- Linear Plasmid Vector
- T4 DNA Ligase Enzyme

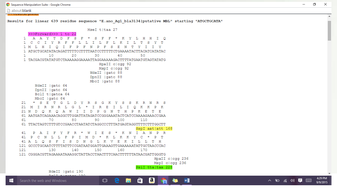
Methods:

The replication of our DNA sequence (>E.ano_Ag1_bla3134|putative MBL) started with translation of the start and stop codons. It only took us a few minutes to replicate the codons, we all compared and ran our results through a sequence reader. The next week we received our DNA forward and reverse primers from Integrated DNA Technologies. We took these and mixed them with the dH2O, 10X Taq buffer, 10X dNTP's, E. miricola or E. anophelis gDNA (58 ng/µl), and Taq polymerase to set up PCR amplification of the genes for cloning. After running through 30 cycles and creating billions of copies our DNA we added the blue dye and inserted the DNA into an electrophoresis tray. Our results came back that we replicated the DNA correct we were supposed to have 639 bases and we did. Then we mixed our DNA encoding protein with HIS-TAG which is like a glue to bond the DNA to the Vector or Carrier DNA. Next was out of our hands because of training purposes and laws. Our TA's and Professor ran some light ligation reactions and came back with a positive on having beta-lactam resistance.

RESULTS

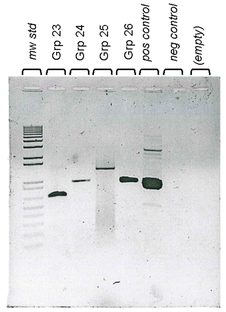
Designing DNA Primers:





At the beginning of the experiment we got a strand of DNA, which need it to be amplified through PCR. We had to meet a certain criteria in order to make sure that we could move to the next phase of the experiment. The forward primers had to start with 5'-ATG and the reverse primers must have had to start with 5'-TTA, 5'TCA, or 5'TCA.

DNA Gel Electrophoresis:



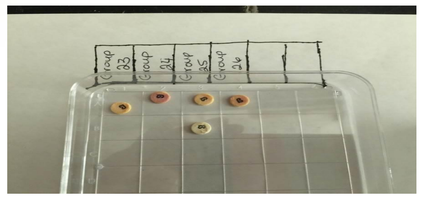
Gel 6

Group	Sample
23	E.ano_bla3134
24	E.ano_bla3289
25	E.ano_bla3306
26	E.ano_bla3533
Pos	E.ano_strep2459
Neg	E.ano_strep2459 no primers

We used this method to separate the different components of our DNA fragment in order to analyze it. This method was used to separate the DNA fragment by length, and therefore estimating the size of these fragments by charge. The agarose was used to move the negatively charged molecules. The shorter molecules move faster and migrate farther than the longer ones because the shorter ones move more easily through the pores of the gel.

Beta-lactamase gene Activity:

We were group 23 so we had a low capacity of resistance for out beta-lactamase gene. This doesn't mean that we didn't follow the right procedures since the beginning. It means that the DNA strand that we got at the beginning had a low capacity for the antibiotic that we tested it for.



DISCUSSION

The purpose of this experiment was to determine which gene or genes in *Elizabethkingia meningoseptica* encode for the enzyme Beta-lactamase. We did this by examining a DNA sequence and designing a primer to use for the cloning process. We used PCR amplification for the cloning process and tested it along the way to make sure it was on the right track. We were successful in producing a cloned beta lactamase gene, proving our hypothesis right. This means that the strand of DNA present in the *Elizabethkingia meningoseptica* gene that we cloned and tested carried the beta lactamase gene. This means that we have found one of the specific stands of DNA that contains the gene that causes *Elizabethkingia meningoseptica* to be resistant to certain antibiotics. Because our gene was proven a real beta lactamase, many more experiments could be done testing the antibacterial resistance of the beta lactamase gene in *Elizabethkingia meningoseptica.* We could tell by the light color of the Nitrocelin qualitative colorimetric assay that our gene was a low capacity beta lactamase, but a true one nevertheless, this just means that it might not be resistant against the antibiotics 100 percent of the time. On a bigger scale, this process can open up doors to find the strands of DNA that contain these genes that make bacteria resistant, which can help in making this gene inactive. This will make sure that bacteria that can cause disease in humans can be stopped by common antibiotics. DNA Gel Electrophoresis:

REFERENCES

1. Mathur, P. (2013, July 25). Elizabethkingia meningoseptica : An emerging pathogen causing meningitis in a hospitalized adult trauma patient. Retrieved November 8, 2015.
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3. EMBL - European Molecular Biology Laboratory. (n.d.). Retrieved November 8, 2015.
4. Wikipedia. (n.d.). Beta-lactamase. Retrieved November 8, 2015.
5. Takashi Shinha, Rakesh Ahuja, Bacteremia due to Elizabethkingia meningoseptica, IDCases, Volume 2, Issue 1, 2015, Pages 13-15, ISSN 2214-2509