Exploring, Cloning, and Characterizing Beta-lactamase Genes in the Bacteria Elizabethkingia *meningoseptica*

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Abstract

There is a prevalent problem with the infectivity of Elizabethkingia *meningoseptica* in immunocompromised individuals. In researching Elizabethkingia *meningoseptica* the research team hoped to discover putative Beta-lactamase enzyme specific genes in the bacterium's chromosome. These enzyme specific genes would need to be characterized by function in order to establish if any genes are functional for the digestion or blocking of antibiotic compounds. Using general scientific procedures such as the Polymerase Chain Reaction and Transformation, these genes can be tested upon several antibiotics to suggest that these genes are functional as antimicrobial resistant genes. With these findings, efforts can begin to look and see whether these genes suggest certain antibiotics are effective against Elizabethkingia *meningoseptica*.

Introduction

Elizabethkingia meningoseptica is a bacillus; gram-negative bacteria, which are notable due to its large resistance to commonly used antibiotics. The methods in which *E. meningoseptica* gets its antibiotic properties is tremendous, however like other gram-negative bacteria, we believe Beta-lactamase genes play a large role in antibiotic resistance. The Beta-lactamases (more specifically in *E. meningoseptica*) are enzymes, which essentially perform multiple digestion and hydrolysis functions in order to render a respective antibiotic obsolete1 . *E. meningoseptica* proves to be a worthy component to research due to its high rate of infectivity when exposed to immunocompromised individuals. When treated with common antibiotics, the bacteria use Beta-lactamase enzymes in order to thrive. The research team is aware that *E. meningoseptica* has Beta-lactamase enzyme specific genes and that they play a large role, however we do not know if they are the key function within *E. meningoseptica*'s extreme antibiotic properties. We believe specific Beta-lactamase genes coincide with specific antibiotics for resistance and we have characterized twenty-three Beta-lactamase genes within *E. meningoseptica*'s genome. In researching these genes we hope to better understand how each gene functions and how they react to particular antibiotics. In order to characterize and to better understand how genes function within single celled organisms like *E. meningoseptica*, the team performed several different methods to isolate and then characterize the bacteria's genes.. The methods are the quickest and easiest way to generally characterize genes in order to look at them further in the future. In characterizing these genes, this will put us one step closer to making this extremely resistant bacteria an obsolete threat in common medicinal practices.

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Methods

In studying *Elizabethkingia meningoseptica*, common genomic isolation practices were used in order to characterize and determine if the Beta-lactamase genes were functional for antibiotic resistance. These methods include: Genomic DNA Isolation, Polymerase Chain Reaction, Restriction Digest, Ligation, Transformation, and a final gene function assay. In prepping the experiment, no living *E. meningoseptica* was handled to ensure our safety throughout the experiment, however we translated the Beta-lactamase genes into a live commercialized DH5α Escherichia coli strand (which contains no Beta-lactamase genes) in order to easily grow and know only our particular genes were what we were seeing as functional. To better understand the DNA replication process we were required to design our own forward and reverse primers for our particular gene we were given.

The Genomic DNA Isolation2 was performed via the QIAGEN Miniprep Protocol (Photo 1). In order to obtain a substantial amount of DNA we spun the microcapsules containing the ordered E. *meningoseptica* strain inside of a room temperature microcentrifuge for five minutes at 8000 rpm. Then we resuspended the compacted bacterial cells in 250 microliters of commercial P1 buffer via the Vortex. 250 microliters of commercial buffer P2 was added and then incorporated by inverting the microcapsule multiple times. *This is now a temperature sensitive solution and must remain below ten degrees Celsius in order to ensure proper isolation*. 350 microliters of commercial buffer N3 was added and mixed immediately by inverting the microcapsule multiple times. The microcapsules were centrifuged again for ten minutes at 13,000 rpm. The supernatant was retrieved by pipetting and then in order to refine further the capsule was centrifuged for one minute at 13,000rpm. The supernatant was discarded and the capsule was washed of initial buffers by adding .75 milliliter commercial Buffer PB and then was centrifuged again for one minute at 13,000rpm. The supernatant was discarded and .75 milliliter buffer PE was added and centrifuged for one minute at 13,000rpm and the supernatant was discarded again. Fifty microliters of distilled water was added and mixed and then one microliter of the DNA fluid was used for the nanodrop to determine the concentration of plasmid DNA. A Polymerase Chain Reaction2 was performed in order to replicate enough plasmid DNA for accurate characterization and confirmed via electrophoresis (Photo 2): Using a standard microcapsule we added - 31 microliters of distilled water - 10 microliters of 5X phusion buffer - 10 microliters of designed forward primer - 10 microliters of designed reverse primer – 1 microliter of our isolated DNA – 1.5 mictoliters of commercial DMSO. *Before adding next two ingredients make sure solution is below ten degrees Celsius* 1 microliter of commercial dNTPs was added and .5 microliters of commercial phusion HF DNA Polymerase was added. The mixture was inverted several times to distribute and the microcapsule was placed in the PCR machine and was ran under the "BetaLac1" setting. Once PCR is finished the product was placed in the freezer until the next process, which is the Restriction Digest2. The PCR product and a standard sized capsule is obtained, inside the capsule we added roughly 45 microliters of PCR product – 20 microliters of distilled water – 10 microliters of commercial 10X MultiCore Buffer diluted to 1X at 100 microliters – 10 microliters of 10X Commercial BSA diluted to 1X at 100 microliters – 7.5 microliters of SaC1 restriction enzyme – 7.5 microliters of BamHI restriction enzyme. The capsule was

placed in a 37 degrees Celsius heat bath and left overnight for digestion. Once the restriction proteins have been digested the Ligation and Transformation2 process can begin. This is where the control DH5α E. coli strain comes into play and with successful propagation we can determine if the Beta-lactamase genes translated are functional for antibiotic resistance. The *E.* coli cells were frozen and we thawed for 2 hours at 25 degree Celsius. 25 microliters of the negative control pSTV28-blab3 gene, and 25 microliters of the positive control pSTV Streptomycin gene were added to two different standard capsules. 2 microliters of the ligated and digested Beta lactamase genes were added to twenty standard capsules. 2 microliters of the suspended E. coli cells were added to all twenty-two capsules and were incubated on ice for thirty minutes. All of the capsules were then heat shocked at 42 degrees Celsius for 35 seconds. All of the capsules were then placed back on ice for 3 minutes. 200 microliters of commercial RTSOC was added and placed in incubator at 37 degrees Celsius for less than 18 hours. After the E. coli cells have had time to incubate, the Gene Function Assay2 can begin (Tables 1 and 2). Each petri dish was created using a low (50 gram) and a high (350 gram) concentration of the six antibiotics: Amoxicillin, Ticarcillin, Cefoxitin, Cefuroxime, Carbenicillin, and Ampicillin. Also two positive controls of Chloramphenicol and a general nutrient agar were created along with the low (50 gram) and high (350 gram) concentration of Streptomycin was created. To create these gels, sterile petri dishes were retrieved and a large amount of agar was created to fill all twenty-three petri dishes. However, when adding the low and high concentration antibiotics each was mixed while the gel was still hot inside of their own beakers. After all plates have been poured and streaked they were incubated for less than 18 hours at 37 degrees Celsius.

Plate Growth

	blaB3		1112		785		1872		Streptomycin*	
Amoxicillin/Clavulanate Potassium (50/350ug/ml)	+	-	+	-	+	-	+	-	+	-
Ticarcillin/Clavulanate (50/350 ug/ml)	+	+	+	-	+	-	+	-	+	-
Cefoxitin (50/350 ug/ml)	+	-	+	-	+	-	+	-	+	-
Cefuroxime sodium (50/350 ug/ml)	+	-	-	-	-	-	-	-	-	-
Carbenicillin (50/350 ug/ml)	+	+	+	+	+	+	+	+	+	+
Ampicillin (50/350 ug/ml)	+	+	+	+	+	+	+	+	+	+
Chloramphenicol (35 ug/ml)	+		+		+		+		+	
Streptomycin (50/350 ug/ml)	-	-	-	-	-	-	-	-	+	+

The table shows positive and negative signs indicating whether there was positive (+) visible colonies on the grown petri dishes or no (-) visible colonies. The numbered columns were the Beta-lactamase genes tested starting with the confirmed blaB3 gene and the isolated genes to the right.

Results

The table shows positive and negative signs indicating whether there was positive (+) visible colonies on the grown petri dishes or no (-) visible colonies. The numbered columns were the Beta-lactamase genes tested starting with the confirmed

blaB3 gene and the isolated genes to the right.

Discussion

Data suggests that the three Beta-lactamase genes characterized and tested were resistant to multiple antibiotics applied to their translated strains. Also, the methods used can be confirmed with the positive growth on the Chloramphenicol plates and the negative growth on the Streptomycin plates. Each Beta-lactamase gene proved to show substantial resistance to the antibiotics except for the Cefuroxime antibiotic. This suggests that Cefuroxime may be a worthy drug to treat *Elizabethkingia meningoseptica*, however much more research needs to be done in order to confirm that evidence and test to see whether that is a viable option. There are also nineteen other confirmed Beta-lactamase genes within our particular Elizabethkingia *meningoseptica* strain and demand characterization as well. As our methods prove to be substantial, some changes can be done in order to fine tune our results into more cohesive data. Establishing gradient plates instead of having absolute high and low concentrations of antibiotics will allow us to see exactly when a concentration of an antibiotic will be effective towards *Elizabethkingia meningoseptica*'s Beta-lactamase genes. Also using different strains of E. coli cell like the KAM-43 strain, which are devoid of efflux pumps (another antibiotic mechanism) will allow us to see whether these genes are functional across multiple platforms of antibiotic resistance. Ultimately, the evidence suggests a promising direction in isolating *Elizabethkingia meningoseptica*'s antibiotic resistant genes for testing. Hopefully with continued research, this research can influence others to look into the medicines required to inhibit the continued spread of this bacteria through immunocompromised individuals.

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Literature Cited

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