Cloning and Characterization of *E. meningoseptica* Beta Lactamase

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Abstract

Elizabethkingia meningoseptica is a rod-shaped bacterium that has been known to cause outbreaks of meningitis, and occasionally sepsis in infants and adults with weakened immune systems. This bacteria has been shown to be resistance to most antibiotics, or antimicrobials, that are used to treat Gram-negative bacterial infections. The purpose of our experiment is to target the predicted genes associated with antibiotic resistance in *E. meningoseptica*, clone the specific gene in this pathogenic bacterium, and characterize the gene to further understand the function. With the published genomic sequence of *E. meningoseptica* ATCC 13253 type strain, we will design primers for PCR amplification of multi-drug RND-efflux pumps specifically for beta-lactamase enzymes, and clone the PCR products into E. coli strain DH5α using the plasmid vector pBBR1MCS-2.n In this experiment, we inserted our gene into DH5α cells, which are a modified *E. coli* used for cloning to look at a specific gene. The gene is inserted into the strain in order to further look at the function of the targeted gene. (E. Coli) We will isolate the recombinant plasmids using a DNA mini-prep method and then confirm the DNA sequence cloned into these plasmids is as expected. We will then screen these clone strains for acquired antimicrobial resistance and degree of acquired resistance. We made antibiotic plates to test the resistance to several different clinically relevant antibiotics.

Keywords: E. meningoseptica, Beta Lactamase, Antibiotic Resistance, Cloning, Gram-negative Bacteria

Introduction

The purpose of this experiment is to further understand the resistance mechanisms of the pathogenic bacterium *Elizabethkingia meningoseptica*. *E. meninoseptica* is found widely spread and are known to cause a breakout of pneumonia, endocarditis, postoperative bacteremia, and meningitis in newborns, infants and susceptible adults. *E. meningoseptica* shows a resistance to the majority of antimicrobials that are used to treat Gram-negative infections. There is genomic sequence data to suggest specific genes associated with antibiotic resistance to *E. meninoseptica*, but there have not been any connections made between the genotype and the observed phenotype. The purpose of this research is to better understand *E. meningoseptica* resistance to antimicrobials by targeting the predicted resistance genes in the *E. menigoseptica* genome, cloning the expressed gene and characterizing the gene. With the published genomic sequence of *E. meningoseptica* ATCC 13253 type strain, we will design primers for PCR amplification of multi-drug RND-efflux pumps specifically for beta-lactamase enzymes, and clone the PCR products into E. coli strain DH5α using the plasmid vector pBBR1MCS-2. We will isolate the recombinant plasmids using a DNA mini-prep method and then confirm the DNA sequence cloned into these plasmids is as expected. We will then screen these clone strains for acquired antimicrobial resistance and degree of acquired resistance. To characterize the antimicrobial resistance, we plan to use concentrated antibiotic plates to look at the growth of the bacteria. Ultimately, we are subcloning *Elizabethkingia meningoseptica* the entire antimicrobial resistance in this manner into E. coli for further analysis, comparison and characterization.

*=Mentor

Figure 1

This table is depicting the methods for this experiment.

Background

Elizabethkingia meningoseptica (formerly known as *Chrysobacterium meningosepticum* and *Flavobacterium meningosepticum*) is a Gram-negative pathogenic bacterium, shown to resist many of the antimicrobials that are used to treat Gram-negative infections(Woodford et al. 2000). While we know that *E. meningoseptica* is resistant to most antimicrobials used to treat Gram-negative infections, connections have not been made between the predicted phenotype and the genotype. *E. meningoseptica* has is known to be found in both freshwater, seawater and soil, causing a range of infections, from meningitis to pneumonia to people with a compromised immunity(Matyi 2014). *E. meningoseptica* has been shown to have up to a 50% mortality rate by infected individuals. Naturally, *E. meningoseptica* is resistant to most forms of β-lactamase including carbapenems (Bellais et al. 2000). In testing *Chrysobaterium meningosepticum*, the spectrum of β-lactamase have been expanded and characterized, and inhibited by clavulanic acid, cefoxitin, moxalactam, and imipenem(Bellais et al. 2000). Seeing as how carbapenem β-lactamase have such a strong resistance to bacterium, carbapenem are used for treatments of gram-negative pathogens. Traces of molecular-class B carbapenemase (BlaB) have been characterized from C. meningosepticum and distinct in this species(Woodford et al. 2000).

Methods

For this experiment, we focused on isolating a specific region of the genome of *E. meningospetica*, and specifically targeting the beta lactamase genes to test their resistance to several different types of clinically relevant antibiotics.

We went through these procedures to test different genes from the twenty two targeted β-lactamase genes within *E. meningoseptica*. In this experiment we were only able to test 2-3 genes.

Results: We were able to successfully characterize two of the genes and test their resistance to several different antibiotics. We tested resistance against Amoxicillin/Clavulanate Potassium, Ticarcillin/Clavulanate, Cefoxitin, Cefuroxiume sodium, Carbenicillin, and Ampicillin, using Chloramphenicol and Streptomycin as a positive and negative control for the bacteria plates. Seeing growth on the plates, we know that our plasmids took to the vector (using DH5α cells) as seen in figure 3. The Chloramphenicol, as mentioned, is the positive control and is expected to have shown growth from all bacteria. The Streptomycin was the negative control showing no growth on any of the genes but the negative control gene (Streptomycin aminoglycoside-6-adenylyl transferase). As shown by the plates and the table, there was

Hypothetical Plate Growth					
	blaB3	1112	785	1872	Streptomycin aminoglycoside-6-adenylyl transferase
Amoxicillin/Clavulanate Potassium	+	+	+	+	-
Ticarcillin/Clavulanate	+	+	+	+	-
Cefoxitin	+	+	+	+	-
Cefuroxiume sodium	+	+	+	+	-
Carbenicillin	+	+	+	+	-
Ampicillin	+	+	+	+	-
Chloramphenicol	+	+	+	+	+
Streptomycin	-	-	-	-	+

Figure 2

This table is showing the expected behavior of the bacteria plates.

obvious resistance to several antibiotics due to the growth on the plates. While there is positive results with the growth of the bacteria on the plates, there is also some peculiarity with the growth of bacteria on the negative control (as seen in Figure 3). The Streptomycin aminoglycoside-6-adenylyl transferase was supposed not supposed to show growth of bacteria. A part from this result, there are two different concentrations being tested for most antibiotics. These specific genes are very unique in their resistance to bacteria, as some bacteria grew on higher concentrations of the bacteria, but the growth was inhibited on lower concentrations. The most prominent example of this phenomena is shown by the all strains (excluding Streptomycin aminoglycoside-6-adenylyl transferase) with the Cefoxitin as seen in figure 3.

This is the hypothetical plate growth. We expected our genes to grow on the antibiotics in order to show their resistance to these drugs. The streptomycin served as a negative control, in which the targeted genes were not to grow on. The chloramphenicol served as a positive control, in which, all the bacteria grew on these plates.

Discussion

In this research, we are testing to see if the β-lactamase genes within *E. meningoseptica* are functional. In order to test the functionality of the β-lactamase genes, we cloned a specific segment of the sequence of *E. meningospetica*, and inserted the gene into the pSTV28 vector and then transformed the plasmid into dh5α cells and

Plate Growth										
	blaB3		1112		785		1872		Streptomycin aminoglycoside-6-adenylyl transferase	
Amoxicillin/ Clavulanate Potassium (50/350ug/ml)	+	-	+	-	+	-	+	-	+	-
Ticarcillin/Clavulanate (50/350 ug/ml)	+	+	+	-	+	-	+	-	+	-
Cefoxitin (50/350 ug/ml)	-	+	-	+	-	+	-	+	-	-
Cefuroxiume sodium (25/350 ug/ml)	+	-	-	-	-	-	-	-	-	-
Carbenicillin (50/350 ug/ml)	+	+	+	+	+	+	+	+	+	+
Ampicillin (50/350 ug/ml)	+	+	+	+	+	+	+	+	+	+
Chloramphenicol (35 ug/ml)	+		+		+		+		+	
Streptomycin (25/200 Ug/ml)	-	-	-	-	-	-	-	-	+	+

Figure 3

This table is showing the actual plate growth in this experiment.

screened on antibiotic plates. From our results, we can claim that the genes are functional, in the fact that they did indeed show resistance to the antibiotic plates. While our modified strains showed antibiotitic resistance, there is odd data that conflicted with completely confirming the functionality of our genes. Due to streptomycin growth on the ampicillin plates, we will move the plasmids to the KAM43 cell line that is devoid of efflux pumps to repeat the experiment. We will also be including two additional negative controls (vector only and cells only).

Further Research

We will be continuing this research with a few modifications. To have a more effective insight as to the exact concentration that the targeted genes grow, we will be using different plate methods. We will be using MICs, gradient plates, and the Kirby Bauer Disk Method to determine a

more definite concentration of the antibiotic will inhibit the biosynthesis of the bacterial cell wall. We will also be inserting are β-lactamase gene into a different *E. coli* strain (KAM43), which is devoid of the efflux pumps. By using this separate strain, we can further understand and characterize the β-lactamase gene. A part from slightly modifying our procedure, we will also be using real time PCR compare the mRNA from the plasmid of *E. coli* to the actual mRNA from *E.meningoseptica,* and more accurately confirm that the protein is actually being synthesized. An important mechanism of an antimicrobial resistance is drug efflux, which are carried out by the genes to perform a function in the process of the drug efflux pump. There are five classifications of efflux: the major facilitator superfamily (MFS), ATP-binding cassette superfamily (ABC), small multi-drug resistance family (SMR), resistance-nodulation-division family (RND), and the multidrug and toxic compound extrusion family (MATE)(Matyi 2014). In the gram-negative family, the RND-type efflux pumps are what are significant in resistance to a broad-spectrum of antimicrobial agents(Matyi 2014). We would like to see if there are efflux pumps involved with the resistance, and would also like to characterize them if they are present.

Acknowledgements

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