Mini-Review of the Literature behind *Elizabethkingia meningoseptica* AND The Cloning and Characterization of *E. meningoseptica* 1872 β-Lactamase

By

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Abstract

Elizabethkingia meningoseptica is an opportunistic pathogen that is classified as Gram negative, rod-shaped bacterium. Notably, *E. meningoseptica* is capable of causing life-threating infections in infants and immunocompromised individuals. This bacterium is resistant to most β-lactam antibiotics that physicians habitually use to treat Gram-negative bacterial infections. In this research project, our aim is to further understand and characterize the specific genes associated with antibiotic resistance in *E. meningoseptica* bacteria; specifically the genes that code for the β-lactamase enzymes capable of altering β-lactam antibiotics and thereby rendering them an ineffective treatment against such infections. In order to combat this multidrug-resistance to antibiotics seen in countless microorganisms such as *E. meningoseptica*, research is needed to understand the function of β-lactamase enzymes and how they contribute to this resistance. Therefore, the purpose of this research project is to investigate the predicted 1872 β-lactamase gene, bla1872, that could be associated with antibiotic resistance in *E. meningoseptica*, clone that gene into expression, and then characterize this gene in order to further understand its function and transcriptional level of regulation.

Introduction

The published genomic sequence of *Elizabethkingia meningoseptica* ATCC 13253^{T} strain shows there are 21 uncharacterized, putative β-lactamase genes that may be associated with the organism's multidrug-resistant phenotype [23]. This research project aims to identify and characterize 1 of the 21 uncharacterized, putative β-lactamase genes encoded by *E. meningoseptica*. In my small subsection research project, we sought to isolate the region of *E. meningoseptica* that contains the 1872 carbapenem β-lactamase, bla1872.

The following subsections will further explain the background and clinical relevance of *E. meningoseptica*, as well as expand upon what β-lactam antibiotics and β-lactamase enzymes are, how they work, and how bacteria may come to exhibit an antibiotic resistant phenotype.

Background:

The genus *Elizabethkingia* (formerly known as *Chryseobacterium* and *Flavobacterium*) is a member of the Bacteroidetes phylum and belongs to the Flavobacteriaceae family [19]. Originally named *Flavobacterium*, this genus was categorized and thereby renamed *Chryseobacterium* in 1994; the re-categorization and renaming to *Elizabethkingia* occurred in 2005 [19, 32]. This bacterial genus derives its name from Elizabeth O. King who, alongside her colleagues, was the first to describe *Elizabethkingia* bacterium associated with infant meningitis in 1958 [20]. Currently, the *Elizabethkingia* genus contains 5 separate species: *E. meningoseptica*, *E. miricola*, *E. anophelis, E. sp. BM10, and E. endophytica* [18, 19, 22].

All *Elizabethkingia* species are classified as rod-shaped Gram-negative bacteria that are non-spore-forming and non-motile [19]. Their colonies are circular, white-yellow in color, yet non-pigmented, semi-translucent, shiny with entire edges, and range in size from 1μm to 2μm [19]. Additionally, good growth is observed on TSA and nutrient agar plates at temperatures ranging from 25-37°C [19].

Elizabethkingia meningoseptica was first isolated and characterized in 1958 from a case of neonatal meningitis, but the journal article regarding their findings was not published until March of 1959 [20]. Most infections of this bacterial strain can be found in a hospital setting; however, this bacterium is ubiquitous and has also been found in soil, freshwater, marine environments, and on domesticated animals [6]. Testing, interpretation, and identification via

diagnostic and ancillary testing should be undergone with caution because *E. meningoseptica* is often misdiagnosed as another *Elizabethkingia* species.

Clinical Relevance and Recent Outbreaks:

Elizabethkingia species are opportunistic pathogens that typically infect neonates and immunocompromised adults. Several different antibiotics have been used to treat such infections. However, most infections caused by *Elizabethkingia* species are unresponsive to habitually used antibiotic therapies because these strains exhibit a multidrug-resistance (MDR) phenotype. This MDR phenotype is strongly selected for as antibiotics are more widely used.

Although ubiquitous in nature, *Elizabethkingia* infections are usually only seen on occasion. Typically, about 5-10 *Elizabethkingia* infections are reported per year in each state of the United States [10]. Normally, these recorded cases resulted in meningitis of neonates; or meningitis, blood infections, or respiratory infections in immunocompromised children and adults [10]. However, the number of *Elizabethkingia* bacterial infections seen in a clinical setting has increased drastically within the last year. According to the Center for Disease Control and Prevention (CDC) website, there have been two separate outbreaks of *Elizabethkingia* bacterial infections in the United States Midwest region since November of 2015; and, as of April of 2016, there have been 59 confirmed cases of *Elizabethkingia* infections in Wisconsin, with 18 of those cases resulting in death [10]. Likewise, Michigan and Illinois both have one confirmed case of an *Elizabethkingia* infection, and both of these cases resulted in the deaths of the patients involved [10]. Notably, the majority of the patients in these reported cases are over the age of 65 and all have underlying health problems that likely put them at an increased risk of contracting bacterial infections [10].

Early identification and treatment for such outbreaks is imperative for the survival of infected patients. In order to determine which species of *Elizabethkingia* is responsible for the outbreak, the CDC utilizes two separate methods to differentiate between the three species: (1) a type of mass spectrometer called MALDI-TOF which is capable of detecting the "protein fingerprints" of the bacterium; and (2) optical mapping [10]. With the use of this technology, the CDC believes that *Elizabethkingia anophelis* is the species behind the recent outbreaks throughout the Midwestern region of the United States [10]. As of April of 2016, the CDC has yet to determine the source of these outbreaks.

B-Lactam Antibiotics:

Antimicrobial agents, which are also called antibiotics, have been used for the past 75 years as a means of combating bacterial infections in humans and domesticated animals [3]. A large majority of these antibiotics function by targeting bacteria cell walls or cell membranes in such a way that they disrupt the cell and diminish its viability by inhibiting bacterial cell wall synthesis; however there are some antimicrobial agents that target a different part of the bacteria—besides the cell wall or call membrane—in order to disrupt the bacteria's cellular functions beyond repair. Although there are several types of antimicrobial agents, β-lactam antibiotics—such as penicillins, cephalosporins, carbapenems, and monobactams—are often the first antimicrobial agent of choice by physicians for the treatment of Gram-negative bacterial infections, and they are also the most relevant for this research project (Table 1) [8, 21]. The effectiveness of these β-lactam antibiotics is waning as bacteria are evolving and utilizing drug-inactivating β-lactamase enzymes; because of this, increasing multidrug resistance is a constant and legitimate concern for medical professionals and researchers that are attempting to develop novel and overall more effective antimicrobial agents that will combat such bacterial infections.

Table 1. Antimicrobial agents used in domesticated animals or humans that target the formation of a cell wall or cell membrane in the infecting bacteria.

Class	Subclass	Target	Use	Examples
β-lactams				
	Penicillins	Cell Wall	Animal	Benzylpenicillin, ticarcillin, amoxicillin +/- clavulanic acid
		Cell Wall	Human	Penicillin, ticarcillin, pipercillin, amoxicillin +/- clavulanic acid, piperacillin +/- tazobactam
	Cephalosporins	Cell Wall	Animal	Cephalexin, cephalothin, ceftiofur
		Cell Wall	Human	Cephalexin, cephalothin, ceftasidime, cefepime
	Carbapenems	Cell Wall	Human	Doripenem, ertapenem, imipenem, cefepime
	Monobactams	Cell Wall	Human	Aztreonam
Glycopeptides		Cell Wall	Animal	Avoparcin
		Cell Wall	Human	Vanomycin, teicoplanin, telavancin
Phosphonic Acid		Cell Wall	Human	Fosfomycin
Lipopeptides		Cell Wall/Membrane	Human	Daptomycin
Peptide antibiotics		Cell Membrane	Human	Polymyxin B, colistin (polymyxin E)
Ionophores		Cell Membrane	Animal	Monensin, salinomycin

These β-lactam antibiotics operate as enzymes that act to disrupt the last step of bacterial cell wall synthesis. Specifically, these antibiotics target proteins called penicillin-binding proteins, also called PBPs, which are involved in the final steps of peptidoglycan synthesis—peptidoglycan molecules are one of the main components in bacterial cell walls [13]. These PBPs are proteins that exhibit a high binding affinity for penicillin and other β-lactam antibiotics and will bind covalently to such molecules [11, 31]. Thus, by binding to these antimicrobial agents, PBPs cannot perform their cellular functions; and, the cell wall synthesis, and thereby the cellular functions, of the bacterial cell are disrupted beyond repair—leading to the death of the microorganism.

PBP is a major component in rod-shaped bacteria (bacilli), but it is only a minor component in sphere-shaped bacteria (cocci) [13]. Additionally, most helical-shaped bacteria have a similar PBP pattern comparable to that of rod-shaped bacteria [13]. However, these PBPs differ between different bacterial strains or mutants.

Antimicrobial resistance mechanisms:

Over the course of evolution, natural selection has selected for bacterial strains that may exhibit one or several mechanisms to overcome antibiotics and demonstrate multi-drug resistance. Fundamentally, all antibiotic resistance mechanisms aim to prevent the antibiotic drug for binding to its target receptor in the infecting bacteria. Examples of these mechanisms include the following: (1) drug efflux, (2) inactivation of antibiotic drugs, (3) changes to the membrane, and (4) altering the cellular targets of antibiotic drugs. The combination of these different antibiotic resistant mechanisms contribute to the complexity of the multi-drug phenotype, which can contribute to the difficulty in treating bacterial infections caused by drug-resistant microorganisms.

E. meningoseptica mainly uses β-lactamase enzymes—which are the most prevalent method utilized by bacteria to combat β-lactam antimicrobials. β-lactamase enzymes are effective at creating an antimicrobial drug resistance because β-lactam antibiotics can be altered and rendered ineffective since these enzymes are capable of cleaving a molecular bond in the antibiotic and therefore opening the β-lactam ring. Once altered, β-lactam antibiotics are no longer capable of binding to their target receptor within the bacterial cell.

Notably, *E. meningoseptica* ATCC 13253^{T} exhibits a multi-drug resistant phenotype and has been shown to be resistant to 19 of the 30 antibiotics listed on Table 2 [7, 9, 20, 24].

Table 2: *E. meningoseptica* ATCC 13253^{T} Antimicrobial Susceptibility

Antibiotic	Susceptibility	Antibiotic	Susceptibility
Amikacin	R	Linezolid	R
Aztreonam	R	Minocycline	S
Cefamandole	R	Neomycin	R
Cefepime	R	Oxacillin	R
Cefoxitin	R	Piperacillin	S
Ceftazidime	R	Piperacillin/Tazobactam	S
Ceftriaxone	R	Quinupristin/Dalfopristin	R
Chloramphenicol	R	Rifampin	S
Ciprofloxacin	S	Spectinomycin	R
Clindamycin	S	Streptomycin	R
Erythromycin	I	Tetracycline	R
Fusidic Acid	S	Ticarcillin/Clavulanic Acid	R
Gentamicin	R	Tigecycline	R
Levofloxacin	S	Trimethoprim/Sulfamethoxazole	S
Lincomycin	R	Vancomycin	S

Abbreviations: R = resistant; S = susceptible; I = intermediate-resistance

Efflux pumps:

One mechanism that microorganisms utilize in order to evade antibiotics is a drug efflux pump. Drug efflux pumps actively transport antibiotic drugs out of the bacterial cell so that these antimicrobial drugs are incapable of binding to their target receptors inside the cell. There are different types of efflux pumps, but they all can be placed into one of five different groups: (1) resistance-nodulation-division family (RND), (2) major facilitator superfamily (MFS), (3) ATP-binding cassette family (ABC), (4) small multidrug-resistance family (SMR), and (5) multidrug and toxic compound extrusion family (MATE) [24].

In Gram-negative specimens, like *E. meningoseptica*, the RND-type efflux pumps are what contribute in their resistance to a wide range of antibiotics [25]. The RND-type efflux pumps consist of three membrane complexes: an outer membrane complex, a membrane fusion

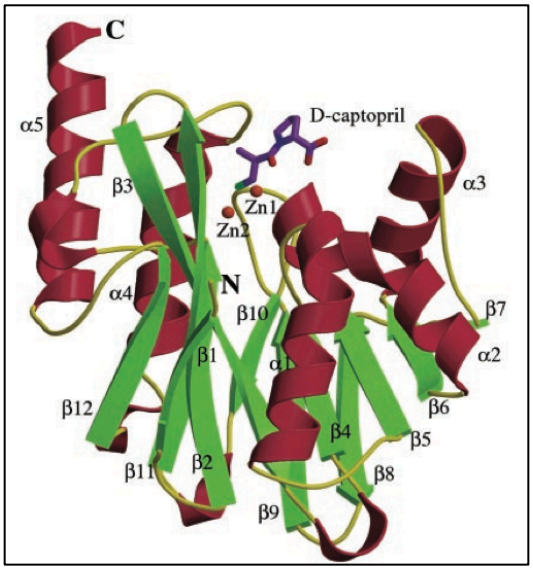
protein, and a resistance-nodulation-division transport protein. [27]. All three of these membranes work together in order to create a proton-drug antiport that can pump the antibiotic molecules out of the bacterial cell [27].

β-Lactamase Enzymes:

Numerous strains of bacteria produce β-lactamase enzymes that can result in a multidrug-resistant phenotype in that bacterium. Essentially, these β-lactamases hydrolyze the β-lactam ring found in all β-lactam antibiotics and thereby inactive these drugs, rendering them ineffective treatments.

In regards to *E. meningoseptica* ATCC 13253^{T}, three β-lactamase enzymes have already been characterized: blaB class B metallo-β-lactamase in 1998 (Figure 1), CME-1 class A serine β-lactamase in 1999, and bla_{GOB} a class B metallo-β-lactamase in 2000 [4, 5, 12, 18, 29]. However, according to the published genomic sequence of *Elizabethkingia meningoseptica* ATCC 13253^{T} strain there are 21 uncharacterized, putative β-lactamase genes that may be associated with the organism's multidrug-resistant phenotype [23].

Figure 1. Crystal structure of BlaB β-lactamase from *E. meningoseptica. In this figure, the enzyme is in complex with D-captopril* [12].



Classifying β-Lactamase Enzymes:

β-lactamase enzymes can be classified and named on the basis of their functional properties and molecular characteristics. There are four different molecular classes that a β-lactamase enzyme can be sorted into: A, B, C, and D [9]. β-lactamase enzymes are typically classified into one of these four classes upon bioinformatics analysis of the bacterial genome; however, these classes are then confirmed using biochemical techniques in the laboratory.

Classes A, C, and D are all serine β-lactamase enzymes that have a conserved serine residue in their active site to coordinate and align their antibiotic substrate in order to successfully hydrolyze the β-lactam ring [9]. Furthermore, Classes A, C, and D β-lactamase enzymes are differentiated depending on their size and conserved, characteristic amino acid sequence motifs. For example, Class C β-lactamases are the largest of the three: they typically have a molecular size greater than 35 kDa, whereas Class A and Class C β-lactamases have a molecular size less than or equal to 31 kDa. Additionally, Class A, C, and D β-lactamases can also be differentiated by examining their characteristic motifs: Class A β-lactamases will have S^{70}TSK, S^{130}DN, E^{166}XXLN, and K^{234}TG motifs; Class C β-lactamases will have S^{64}TSK, Y^{150}AN, and K^{315}TG motifs; Class D β-lactamases will have S^{70}TFK, S^{118}XV, Y^{144}GN, and K^{216}TG motifs [1, 2, 5, 14, 15, 16, 17, 26, 30].

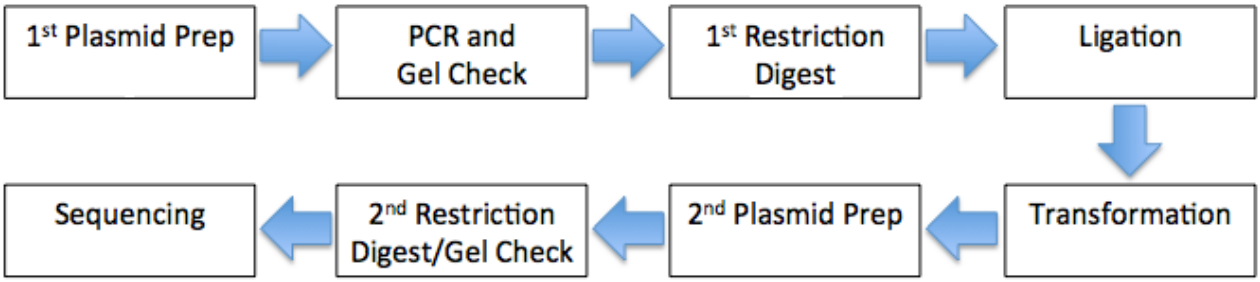
Class B β-lactamase enzymes are all metallo-β-lactamases that have 1 or 2 Zn^{2+} atoms at their active site to coordinate and align their antibiotic substrate in order to successfully hydrolyze the β-lactam ring [9]. Additionally, there are 3 different subclasses within Class B β-lactamases; but since our putative β-lactamase for this research project does not fall under Class B, we will not expand on its subclasses.

The β-lactamase we are manipulating in this research project, bla1872, is a putative Class C serine-β-lactamase. Class C β-lactamases are considered cephalosporinases because they actively bind and cleave the β-lactam ring in cephalosporin antibiotic drugs [9].

Materials and Methods

For this experiment, we ended up focusing our attention on isolating and cloning the specific region of *E. meningoseptica* that contains the 1872 carbapenem β-lactamase, bla1872. I was given a preliminary plasmid (see below) that contained the coding sequence, plus 500 bases up and downstream, from which we wanted to transfer the coding sequence into a new vector. By taking the original plasmid and creating a new clone, we will then be capable of controlling the induction of the promoter for the bla1872 gene with the use of IPTG. The steps we used to obtain our eventual pSKB3-Em1872 clone are outline below (Figure 2).

Figure 2. Procedural steps used to obtain the pSKB3-Em1872 clone.



Material:

I was assigned the pSTV28-Em1872 plasmid at the beginning of this research project.

1st Alkaline Lysis Plasmid Prep:

We first centrifuged the pSTV28-Em1872 bacterial culture sample. The supernatant was discarded and the bacterial pellet was kept. We then added 100.0 µL of Solution I (Tris buffer and RNase) to tube containing the pellet and subsequently suspended the pellet with the use of a

vortex. Next, we added 200.0 μL of Solution II (SDS, NaOH) to the tube and mixed the contents by gentle inversion. Similarly, 150.0 μL of Solution III (Potassium Acetate) was then added to the tube and also mixed by inversion. The sample was then centrifuged and the supernatant was removed and placed into a new tube; the pellet was discarded. Once the supernatant was placed into a new tube, 500.0 μL of 95% ethanol was added, and then contents were again mixed by gentle inversion. Our sample was then placed in the freezer at -20°C overnight.

The following day, the samples were centrifuged. The pellet that resulted was kept, and the supernatant was discarded. Next, 500.0 μL of 70% ethanol was added and the sample was then centrifuged once more. Once again, the supernatant was then discarded and the pellet was kept. Subsequently, 50.0 μL of sterile H_2O was added to the sample. Finally, the sample was placed in a water bath at 37°C for 10 minutes and then returned to freezer.

PCR and Gel Check:

Polymerase chain reaction (PCR) amplification was used in order to increase the amount of the bla1872 coding sequence we had to manipulate. Three separate tubes, with different combination of reagents, were prepared and then allowed to undergo PCR (Table 3).

After amplification was complete, a 1% agarose gel was made with 50.0 mL of TBE and 0.50g of agarose powder. Samples from each of the three were loaded alongside a 1 Kb+ DNA ladder in order to verify that the bla1872 coding sequence was amplified correctly.

Table 3. PCR set-up/reagent amounts used.

	Tube 1	Tube 2	Tube 3
dH_2O	31.0 µL	31.0 µL	29.5 µL
5X phusion buffer	10.0 µL	10.0 µL	10.0 µL
10mM dNTP	1.0 µL	1.0 µL	1.0 µL
pSTV28-Em1872 plasmid	1.0 µL	1.0 µL	1.0 µL
10µM Fwd Primer	2.5 µL	2.5 µL	2.5 µL
10µM Rev Primer	2.5 µL	2.5 µL	2.5 µL
50.0 mM $MgCl_2$	0.0 µL	1.5 µL	1.5 µL
DMSO	1.5 µL	0.0 µL	1.5 µL
Phusion DNA taq	0.5 µL	0.5 µL	0.5 µL

1st Restriction Digest:

All three tubes of PCR product were combined, and water was added to the sample in order to reach a total volume of 500.0 µL. Next, 500.0 µL of phenyl-chloroform-isoamyl alcohol was added. Then the sample was placed on a rocker, centrifuged, and the aqueous phase was removed and placed into a new tube. 500.0 µL of chloroform-isoamyl alcohol was added to the sample, it was placed on a rocker, and then centrifuged. Once the second aqueous phase was removed and placed into a new tube, 1.0 µL of glycogen blue and 1.0 mL of 95% EtOH was added to the sample and placed in the freezer at - 20°C.

The following day, the thawed sample was centrifuged and the supernatant was discarded. 500.0 µL of 70% EtOH was added to the pellet and the sample was centrifuged again, with the supernatant being discarded. Our PCR pellet was then re-suspended in 30.0 µL of sterile H_2O and our 1st restriction digest was set-up (See Table 4). Finally, the digest tube was placed in a 37°C water bath overnight. The next morning the sample was returned to the freezer.

Table 4. Restriction Digest #1 Set-up/Contents.

dH2O	44.5 µL
10X cutsmart buffer	7.0 µL
bla1872 PCR product	15.0 µL
Bam HI-HF	1.75 µL
SacI-HF	1.75 µL

Ligation:

In order to set up our ligation, we first had to clean up our digest product. Water was added to the sample in order to reach a total volume of 100.0 µL. Next, 500.0 µL of PB buffer was added and our sample was loaded into the DNA cleanup column. Once in the column, the sample was centrifuged and the flow-through liquid was discarded. Then, 750.0 µL of PE buffer was added and the same was again centrifuged, with the flow-through liquid being discarded. The DNA sample was the eluted off the column with 30.0 µL of sterile H_2O.

Once the bla1872 DNA sample was purified, our ligation was set-up (Table 5) and allowed to sit at room temperature overnight.

Table 5. Ligation Set-up/Contents.

dH2O	11.0 µL
10X ligation buffer	2.0 µL
bla1872 digest sample	4.0 µL
T4 DNA ligase	1.0 µL
SKB3 vector	2.0 µL

Transformation:

To set up our transformation, 2.0 µL of our ligation solution was added to 20.0 µL of our competent cells (*E. coli* DH5α) and allowed to incubate on ice for 30 minutes. Next, we heat-shocked our sample for 30 sec. in a 42° C water bath and the sample was then immediately placed back on ice. Subsequently, 200.0 µL of SOC media was added to the sample and it was

placed in the shaker at 37° Celsius for 2 hours. Finally, 100.0 µL of the sample was plated onto a kanamycin agarose plate and allowed to grow overnight in the incubator set at 37°C.

Ten colonies were selected the next day and placed into separate tubes containing LB broth with kanamycin. Samples were allowed to grow overnight in the incubator set at 37°C.

2nd Alkaline Lysis Plasmid Prep:

The same steps used in the 1st Alkaline Lysis Plasmid Prep were also used in this step (see above). However, instead of using pSTV28-Em1872 bacterial culture, we used the selected colonies from our transformation that hopefully contain the pSKB3-Em1872 clone.

2nd Restriction Digest/Gel Check:

A second restriction digest was set up in order to determine if we had isolated the pSKB3-Em1872 clone (see Table 6). The 2nd digest tube was placed in a 37°C water bath overnight and then placed in the freezer at - 20°C.

Table 6. Restriction Digest #1 Set-up/Contents

dH2O	9.0 µL
10X cutsmart buffer	2.0 µL
pSKB3-Em1872 plasmid	5.0 µL
XhoI	2.0 µL
XbaI	2.0 µL

Next, a 1% agarose gel was made with 50.0 mL of TBE and 0.50g of agarose powder. The sample from the digest solution was loaded alongside a 1 Kb+ DNA ladder in order to verify that the pSKB3-Em1872 clone was transformed correctly.

Sequencing:

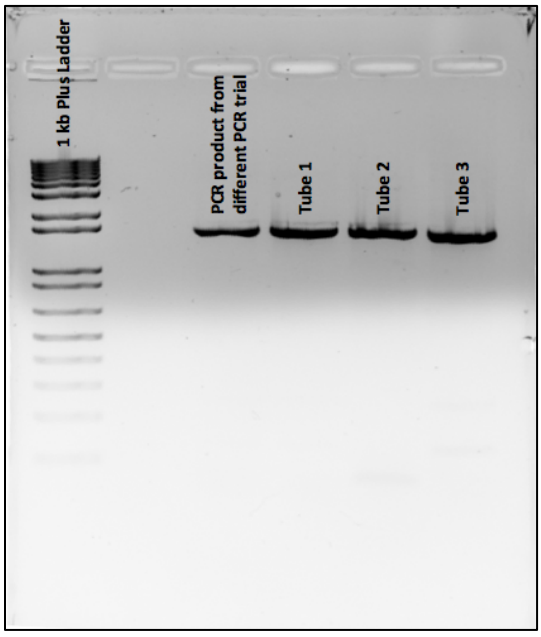
In order to verify that we did indeed isolate the pSKB3-Em1872 clone, a 20.0 µL DNA sample of our pSKB3-Em1872 clone was sent to the OSU Biochemistry and Molecular Biology

Recombinant DNA and Protein Core Facility alongside the clone's T7 promoter and 7T terminator. Finally, we will know if we created our new clone by comparing the genomic sequence of our putative pSKB3-Em1872 clone and the published genomic sequence of *E. meningoseptica* ATCC 13253^{T} [23].

Results

In order to verify that the bla1872 coding sequence was amplified correctly, a 1% agarose gel was loaded with all 3 of our PCR samples alongside a 1 Kb+ DNA ladder. The bla1872 coding sequence is approximately 1545 base pairs (bp), and we see this same size band in the lanes containing the PCR contents of Tube 1, Tube 2, and Tube 3—which indicated that we amplified our DNA successfully (Figure 3).

Figure 3. Post-PCR agarose gel. Bands at approximately 1545 bp indicate that we successfully amplified the pSTV28-Em1872 DNA.



In order to determine if our pSKB3-Em1872 plasmid was successfully obtained, a 1% agarose gel was loaded with all 10 of our Restriction Digest #2 samples alongside a 1 kb plus

DNA ladder. Because we cleaved the pSKB3-Em1872 plasmid with at XhoI and XbaI, we expect to see two bands at approximately 5200 bp and 1700 bp if our transformation was successful. In lanes contain Tube 7 and Tube 9 we see two bands at 1700 bp (the bla1872 insert) and 5200 bp (the remaining *E. coli* DNA)—which indicates that we amplified our obtained a putative pSKB3-Em1872 clone (Figure 4).

Finally, upon comparing the genomic sequence of our putative pSKB3-Em1872 clone alongside the published genomic sequence of *E. meningoseptica* ATCC 13253^{T}, we were able to determine that we were successful in creating our desired pSKB3-Em1872 clone (Figure 5) needed for the future research goals of this project.

Figure 4. Post-Restriction Digest #2 1% agarose gel to screen for potential pSKB3-Em1872 clones. LEFT: The lanes of Tube 7 and Tube 9 (barely visible on this picture) show two separate bands at approximately 5200 bp and 1700 bp. RIGHT: Repeated Post-Restriction Digest #2 1% agarose gel on Tube7 and Tube 9 to verify that there were bands at approximately 5200 bp and 1700 bp on a full scale gel.

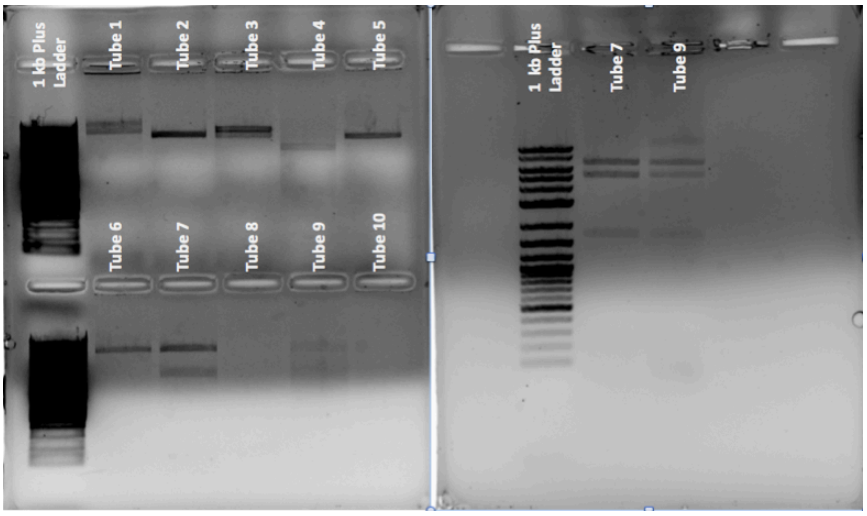
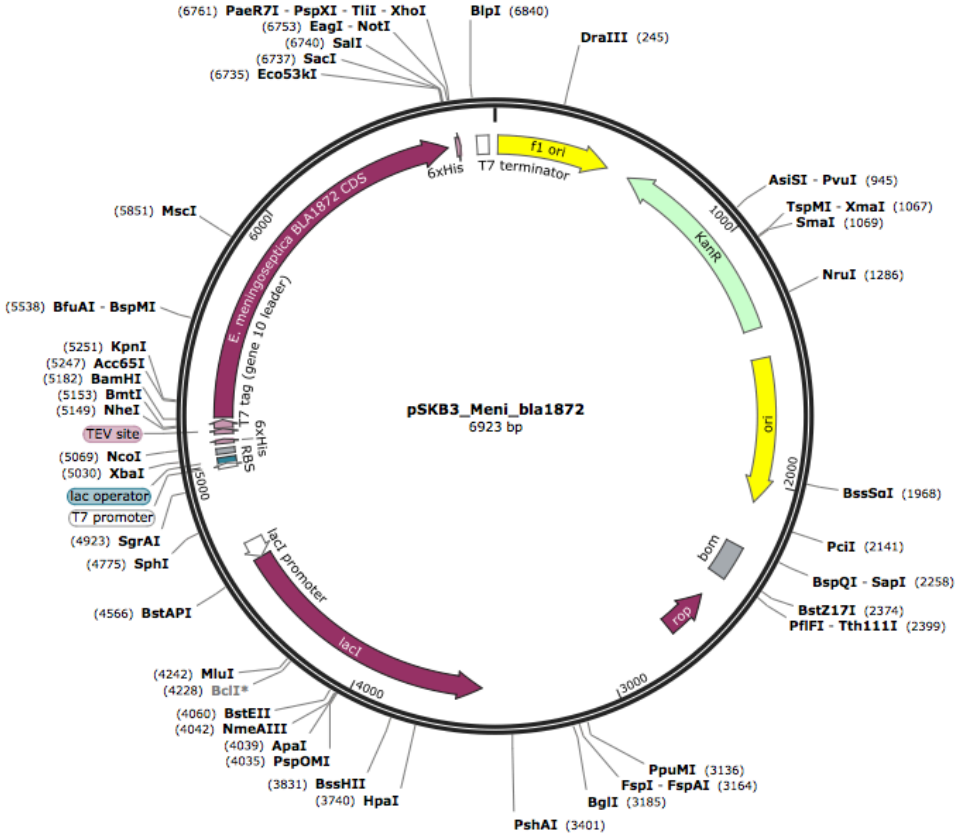


Figure 5. Plasmid map of the verified pSKB3-Em1872 clone obtained.



Future Direction

After successfully cloning the bla1872 β-lactamase gene of *E. meningoseptica* into the BL21 DE3 *E. coli* strain, my successors will then screen this clone against several β-lactam antibiotics via a Kirby-Bauer Disk Susceptibility and a MIC assay. The Kirby-Bauer test will determine bacterium resistance to each individual antibiotic. The MIC assay will be completed in order to determine the antibiotic concentration range in which a bacterium exhibits resistance. The Kirby-Bauer test and the MIC assay will demonstration if bla1872is a true β-lactamase capable of hydrolyzing β -lactam antibiotics. Both the Kirby-Bauer test and the MIC assay are further explained in individual sections below.

Once we determine if it is a β -lactamase we then want to purify and characterize the enzyme. Our clone will be grown and induced to produce the bla1872 β-lactamase gene of *E. meningoseptica*. This bla1872 product will then undergo protein purification, and, once purified the bla1872 enzyme will be characterized via enzyme kinetics in order to meet the requirements for publication.

Kirby-Bauer:

A Kirby-Bauer test is a microbiologic method used to determine a microorganism's sensitivity to an antimicrobial agent. In this method, nutrient agar plates are prepared with the desired antibiotic. Then, the bacteria to be screened is spread on the agar plate, and allowed to grow—often overnight—at the optimal temperature of growth for that specific specimen. Upon examining the colony growth on the agar plate, there should be a zone of inhibition; this is the area of the media where the microorganism is incapable of growing because the antibiotic is effective at combating the pathogen. The size of this zone of inhibition reveals the microorganism's degree of sensitivity to the concentration of antibiotic used: the bigger the zone

of inhibition, the more sensitive it is to that antibiotic; the small the zone of inhibition, the more resistant it is to that antibiotic.

Since bla1872 is a putative group 1, class C β-lactamase that has a high binding affinity and capability of hydrolyzing cephalosporins, we will screen bla1872 against this type of β-lactam antibiotic. Specifically, by screening our bla1872 clone against these antimicrobial agents, our aim in is to determine its specific degree of required resistance against numerous clinically relevant β-lactam antibiotics. We expect to see smaller zones of inhibition in the plates containing our pSKB3-Em1872 clone with its induced bla1872 β-lactamase when compared to our positive control Bla-B (one of *E. meningoseptica*'s already characterized metallo-β-lactamase enzymes) which should show larger zones of inhibition.

MIC:

The Minimum Inhibitory Concentrations assay (MIC) is a test used to determine the lowest concentration of antibiotics that is needed to inhibit a microorganism's growth after an overnight incubation period. Whereas the Kirby-Bauer test will tell us if a microorganism is resistant at a certain concentration of antibiotic, the Kirby-Bauer gives us the concentration range in which a bacterium is resistant to a certain antimicrobial agent.

Conclusion

Our ultimate goal in this research project is to eventually clone, express, isolate, and characterize the bla1872 β-lactamase enzyme of *E. meningoseptica*, in addition to the other punitive β-lactamase enzymes that have yet to be cloned and characterized. Although we were only able successful in obtaining the required clone during my year in the laboratory, my successors will continue this project to characterize the bla1872 β-lactamase enzyme, with the hopes of a publication at the completion of this project.

In the grand scheme of things, we hope that this research project will help fill the knowledge gap regarding β-lactamase genes in *E. meningoseptica* so as to provide a more comprehensive understanding of bacterial-mediated antibiotic resistance in this bacterium, as well as other bacteria that exhibit similar multidrug-resistance via Type 1, Class C β-lactamase enzyme. Although more research is needed to understand these β-lactamase enzymes and how to combat their function in multidrug-resistance, we are optimistic that the knowledge acquired in this research project will assist in the development of more effective antibiotic drugs in the future.

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