β-Lactamases in the multidrug resistance opportunistic bacterial pathogen *Elizabethkingia anophelis*

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Abstract: Beta-lactamases are bacterial enzymes that pose a serious threat within the healthcare field due to their antibiotic resistance towards commonly prescribed antibiotics, including penicillins, cephalosporins, and cephamycins. *Elizabethkingia anophelis*, originally discovered from the *Anopheles* mosquito gut, is the most healthcare relevant species of *Elizabethkingia* due to the severity of the diseases it causes in humans, including meningitis and respiratory infections in populations with weakened immune systems. The work presented here is the beginning stages of identifying unknown beta-lactamases within *E. anophelis*. In this study, we have examined the genome of *E. anophelis* R26 and found 3 known beta-lactamases and 17 additional putative beta-lactamases that we will use for further identification testing. In addition, we have started the cloning process with the native promoter in each of the putative beta-lactamases.

Keywords: *Elizabethkingia anophelis*, Beta-Lactamases, Multidrug Resistance, Putative Beta-Lactamases

Introduction

Elizabethkingia, a genus of gram-negative opportunistic bacteria, is commonly found in natural environments, such as water sources and soil, while also being found in more civilized spaces, such as hospital settings (Lau et al. 2017; Vaneechoutte et al. 2015). *Elizabethkingia* is most successful at infecting hosts with compromised immune systems, and most cases of *Elizabethkingia* are seen in the infant and aging populations. Currently, there are six known species, but *E. anophelis* is the most relevant in healthcare today (Han et al. 2015). *E. meningoseptica*, the first *Elizabethkingia* discovered in 1958 by Elizabeth King, causes the membrane lining of the brain to become inflamed, which is fatal if not caught and treated early enough (Weaver et al. 2015). *E. meningoseptica*'s ability to survive for extended amounts of time in moist environments and antibiotic resistance makes the bacteria an increasing problem in many intensive care units (ICUs), where all of the patients are admitted due to severe medical problems and weak immune systems (Balm et al. 2013). In 2016, an outbreak of *E. anophelis* occurred in Wisconsin – a total of 67 people were infected with the bacteria and 19 of those people died (Wisconsin Department of Health Services 2016). The majority of those infected during the Wisconsin *E. anophelis* outbreak were older than 65 years and had an underlying health condition that suppressed their immune system (Wisconsin Department of Health Services 2016). Later, the outbreak was spread between three states in total (Wisconsin, Illinois, and Michigan), but the source of the outbreak is still unknown despite state and federal investigations (Castro et al. 2017).

While the bacteria rarely results in illness, the vast antibiotic resistance properties all of the species possess against popular antibiotics (beta-lactams, aminoglycosides, tetracycline, and chloramphenicol) makes it more difficult to treat than other bacteria related cases (Shaikh et al. 2015). This project is aiming to identify predicted but uncharacterized beta-lactamases within the *E. anophelis* species so that it can be biochemically characterized for a greater understanding.

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Methods

In order to narrow down the amount of potential beta-lactamase genes to the most likely candidates, certain components, such as the operonic state, protein sequencing, and domains, of the genes were evaluated in a gene selection process.

The first step for gene analysis was to obtain the complete *Elizabethkingia anophelis* genome sequence from the NCBI Blast website (Kukutla et al. 2013). From the general genome sequence, further annotation can be done to find the open reading frames (ORFs), genes, and identify the protein and function. By using the RAST database, all of the *E. anophelis* genes can be organized into a database of genome sequences to check for operons and ORFs (Aziz et al. 2008).

Then, to find putative beta-lactamases, the BlastP tool from the Beta-Lactamase Database was utilized to compare the protein sequencing of putative genes to known beta-lactamase protein sequences (Naas et al. 2017).

Results

Concluding the NCBI Blast search, the *E. anophelis* genome sequencing report showed a total of 4,032,720 base pairs of DNA. Selecting putative beta-lactamase genes starts with finding the overall DNA sequence, which can be broken down into smaller segments for characterization and analysis. From the DNA sequence, the RAST search identified a total of 3963 genes. These genes were then analyzed for their operonic state – given that the majority of known beta-lactamases seem to be non-operonic, we looked at the RAST results to find mostly non-operonic putative beta-lactamases. Because one of the known beta-lactamases, ORF3705, looked to be slightly operonic, we made an exception for its counterpart, ORF3793, due to the similarities they shared in other aspects of the required criteria. After using the Beta-Lactamase Database (BLDB) tool for BlastP analysis, three genes are known beta-lactamases and 32 genes show potential to be a beta-lactamase.

From the 35 genes identified, the NBCI Conserved Domain analysis tool identified 1 known serine-beta-lactamase and 2 metallo-beta-lactamases, along with 2 putative metallo-beta-lactamases and 30 putative serine-beta-lactamases. Of the 32 potential beta-lactamase genes identified from the BLDB analysis, 14 did not result in a located canonical signal peptide from the SignalP 5.0 search, so those genes were not used for further analysis due to the necessity of the signal peptides in beta-lactamases. Finally, the 18 remaining genes were analyzed for native promoters 500 base pairs upstream of the open reading frame. For quantitative analysis of the BPROM search results, we looked for genes with higher promoter scores, as the higher scores typically means a promoter

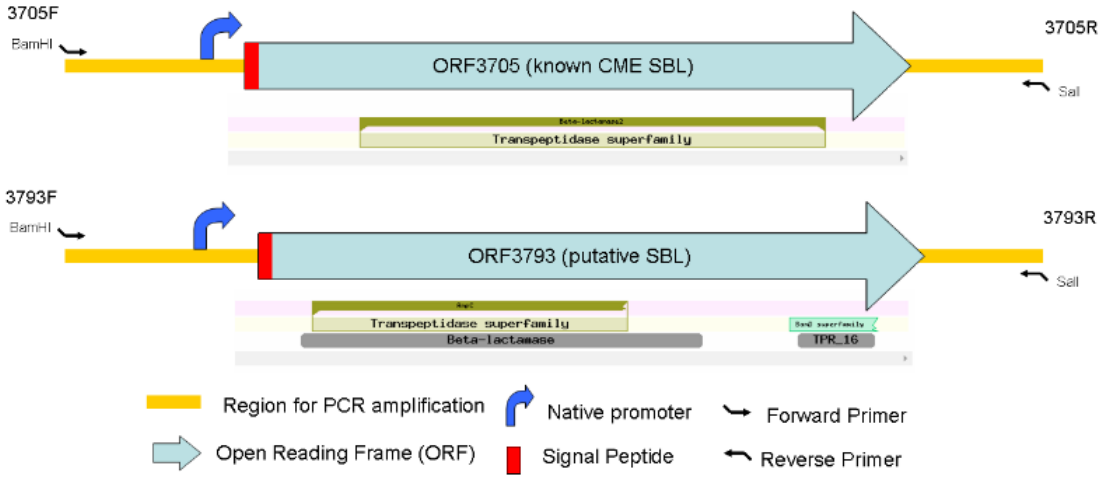

Figure 1: A comparative map between two serine-beta-lactamases – the known beta-lactamase (ORF3705) and the putative beta-lactamase (ORF3793). This map shows the regions that will be used for amplification and cloning, including the ORFs, forward and reverse primers, native promoters, and signal peptides.

is present. Only nine of those 18 putative beta-lactamases resulted in a native promoter, and because previous studies showed success with using native promoters in cloning experiments, we chose to use only genes with native promoters for cloning analysis.

Discussion

In the future, this project will focus on cloning the predicted beta-lactamases of *E. anophelis* R26 strain into the pHSG298 cloning vector. Because we are using the native promoter (which has shown success in the three known beta-lactamases), we will select directly onto ampicillin plates or liquid media in order to test the relative resistance to antibiotics. Slight adjustments will be made in our cloning technique as we progress further towards our goal in order to best suite the bacteria and cloning host. We found the protein of ORF3793, which was part of the project focus this semester, has a probable outer membrane transmembrane domain at the C-terminal end (Figure 1) which could explain some of the cloning difficulties we encountered. In our next cloning attempts, we will select ORFs that lack a transmembrane protein to increase our chances of cloning success.

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