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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 (1). *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 (2), including meningitis and respiratory infections in populations with weakened immune systems (3).

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. In addition, we have started the cloning process with the native promoter in each of the putative beta-lactamases.

BACKGROUND

Elizabethkingia, a genus of gram-negative 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 (3, 4). *Elizabethkingia* is the 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 (5). *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 (6). *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 (8). 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 (7). 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 (7). 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 (9).

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 (3). 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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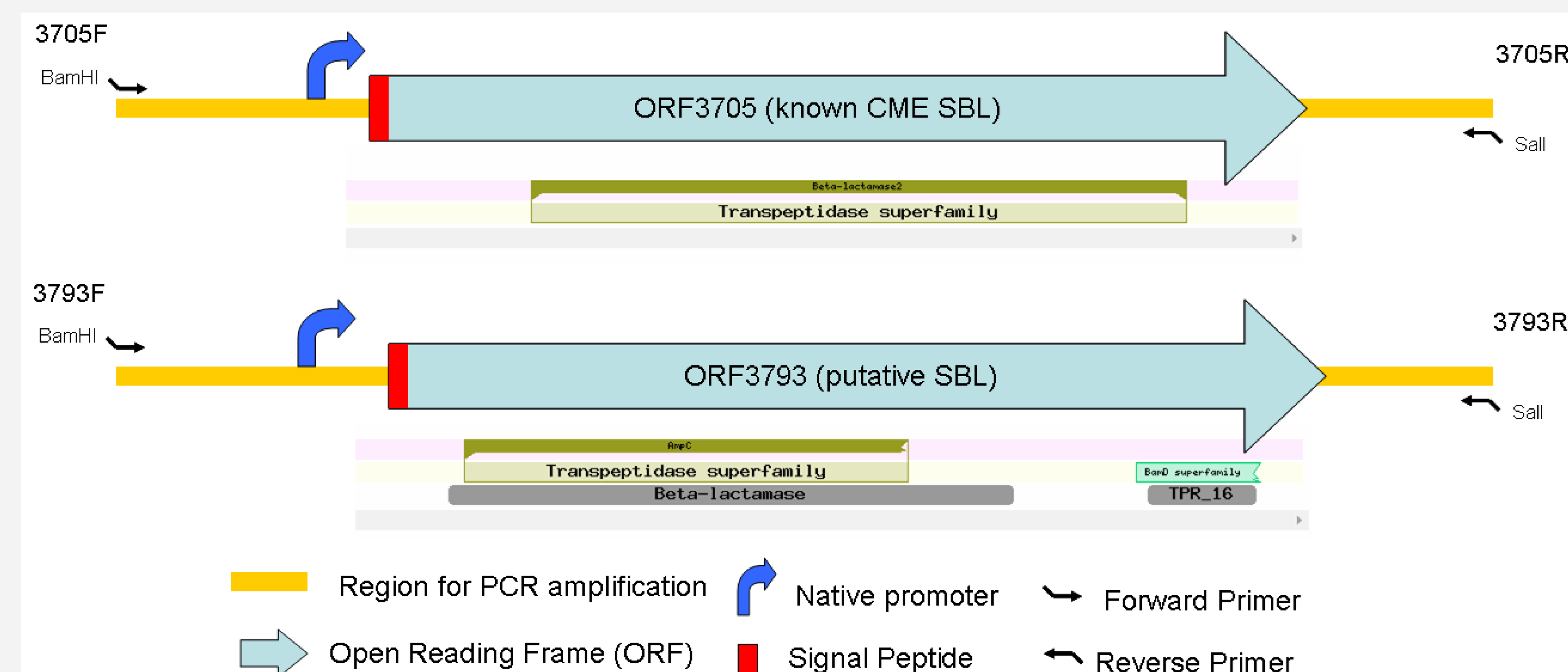
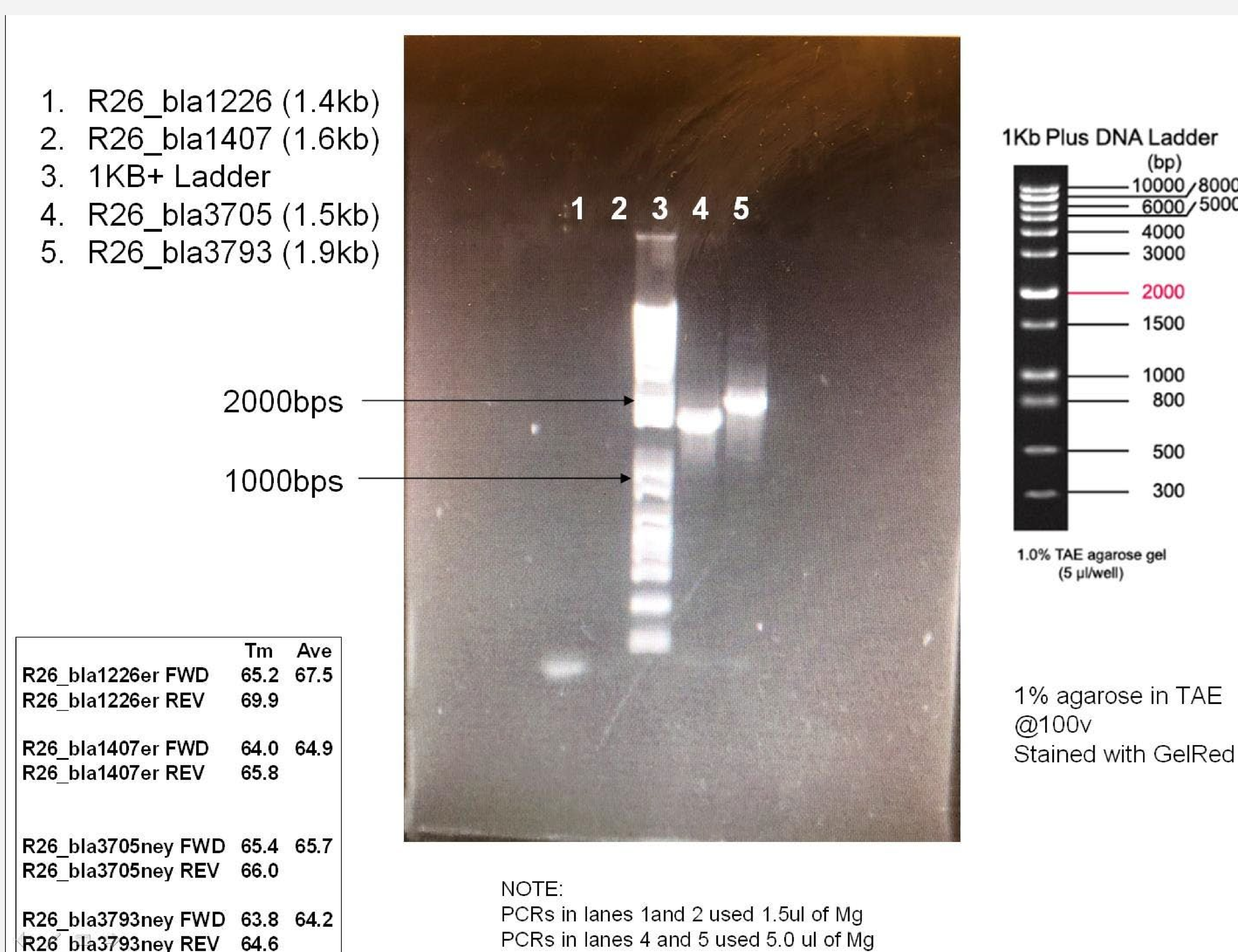


Figure 1. The graphic visualization comparative map of SBL regions used for cloning including primers, promoters, signal peptides, ORFs, and reverse primers. This region was selected for amplification and cloning.



	Tm	Ave
R26_bla1226er FWD	65.2	67.5
R26_bla1226er REV	69.9	
R26_bla1407er FWD	64.0	64.9
R26_bla1407er REV	65.8	
R26_bla3705ney FWD	65.4	65.7
R26_bla3705ney REV	66.0	
R26_bla3793ney FWD	63.8	64.2
R26_bla3793ney REV	64.6	

Figure 2. Our initial PCR amplification of the beta-lactamases. Lane 4 demonstrates the PCR of my known beta-lactamase, acting as a control in our cloning technique. Lane 5 shows the PCR of my unknown beta-lactamase, which is the gene that I am trying to clone for antibiotic resistance testing.

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 show 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 to best suite the bacteria and cloning host. We found the protein of ORF3793, which was my main focus this semester, has a probable outer membrane transmembrane domain at the C-terminal end (shown in Figure 1) which could explain some of the cloning difficulties. In our next cloning attempts, we will select ORFs that lack a transmembrane protein to increase our chances of cloning success.

I would like to thank Dr. Patricia Canaan, who has been such an excellent mentor, in the lab and in life, and my research partner Hunter Tollivar, for helping me learn some of the biochemical basis while also helping me master my pipetting techniques. Also, I would love to extend a big thank you to HHMI for sponsoring freshman research and giving us the opportunity to expand our educational horizons.

METHODS

How we chose the gene (bioinformatics):

- We started with *E.anophelis* genome from NCBI Blast website (https://www.ncbi.nlm.nih.gov/nuccore/NZ_ANIW00000000.1).
- We used RASTbd to create a database of genome sequence from NCBI to identify ORFs and operons (http://rast.nmpdr.org/rast.cgi?page=Jobs).
- Then we used the Beta-Lactamase database to find putative beta-lactamases based on protein sequence similarity to known beta-lactamases (http://www.bldb.eu:4567/).
- We utilized the NCBI Conserved domain analysis tool to check the protein sequence and determine if the genes had beta-lactamase-like domains and to categorize each as either a metallo-beta-lactamase or a serine-beta-lactamases (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi?).
- Then we checked each for N-terminal signal peptide sequences using the SignalP 5.0 website (http://www.cbs.dtu.dk/services/SignalP-5.0/).
- Finally, we checked and identified promoters (500 bp upstream of the gene) using the BPROM program through Soft Berry. We were looking for a high promoter score as higher score typically mean a promoter is more likely (http://www.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb).

RESULTS

Results from the gene selection process:

- From the NCBI search, We found 4032720 base pairs of DNA.
- The RAST search identified 3963 total genes, most likely candidates were non-operonic, but because one known B-Lac looked like it was in an operon, we chose to select those that met all other requirements.
- From the BLDB blastp analysis, we found 32 putative and 3 known beta-lactamases.
- NCBI Conserved domain analysis suggested that there are 4 metallo-beta-lactamases (2 previously known) and 31 serine beta-lactamases (1 previously known).
- The signal peptide sequence analysis revealed 14 of 31 potential beta-lactamases lack a canonical signal peptide and those were not used in further analysis.
- It is necessary that each gene have its own promoter for cloning success and BPROM was used to search for possibly promoters upstream of the beta-lactamase ORFs. Following this step, we were left with 9 putative beta-lactamases.