Five Heat Shock Proteins and Their Abundance of Transcriptions in *E. anophelis* R26 in Three Different Conditions

GRP# 26

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ABSTRACT

Elizabethkingia anophelis is an antibiotic resistant bacteria causing life threatening disease in people across the United States. In order to understand how it is resisting antibiotic treatment, we selected five heat shock proteins from *E. anophelis* and compared their number of transcript levels under two different antibiotics: Cefotax and Imipenem. We looked for significant changes in the level of transcripts from the control to each condition. For gene #1326, a significant change of 1.5 occurred when the gene was exposed to Cefotax. Gene #209 had no significant changes in either antibiotic. When exposed to Cefotax, gene #223 significantly changed by -3.5. When exposed to Imipenem, gene #223 significantly changed by 1.6. Gene #2968 significantly changed by -2.0 when exposed to Cefotax and by 2.5 when exposed to Imipenem. Gene #1796, when exposed to Cefotax, significantly changed by 1.9, and by -1.6 when exposed to Imipenem.

INTRODUCTION

Elizabethkingia anophelis is a microbe that causes human disease, such as meningitis. It has been brought to the attention of the public after recent outbreaks throughout the Midwest. From 2015 to 2016 in Wisconsin, 63 people were infected and 18 died (4). What makes this bacteria so dangerous is its resistance to antibiotics (2). In an attempt to find a way to combat this organism, we determined the transcription levels within *E. anophelis* when it was exposed to the antibiotics Cefotex and Imipenem. *E. anophelis* is an complicated bacteria with 4,369,828 base pairs and 4,141 coding sequences (3).

We focused on 5 specific proteins, all related to how the bacteria is affected by stressful stimuli, specifically heat shock. The proteins were gene #1326 tmRNA-binding protein SmpB, gene #209 Chaperone protein DnaK, gene #223 Ribosome-associated heat shock protein implicated in the recycling of the 50S subunit (S4 paralog), gene #2968 Nucleoside 5-triphosphatase RdgB (dHAPTP, dITP, XTP-specific) (EC 3.6.1.15), and gene #1796 Heat shock protein GrpE. Heat shock proteins are expressed during stressful conditions such as heat shock. These proteins help proteins stay folded correctly so that they do not lose their function (6).

MATERIALS AND METHODS

1) We began by navigating to the RAST database (1). There we chose five heat shock proteins.
   1) Our five genes are genes #1326, #209, #223, #2968, and #1796.
2) We then searched the BLAST site (5) to determine if our genes were transcribed when *E. anophelis* was exposed to the control, Imipenem, and Cefotax.
3) Following, we used the excel sheet given - E.anoR26_RNAseqData_FoldChanges - that showed the number of transcripts for each gene in each condition. We used this spreadsheet to determine if the changes in transcriptions were significant. A significant change occurred if the fold change was ⩾1.5, or ⩽-1.5.
4) The final step was to use the RAST database (1) to determine if any of our genes of interest were in operons.

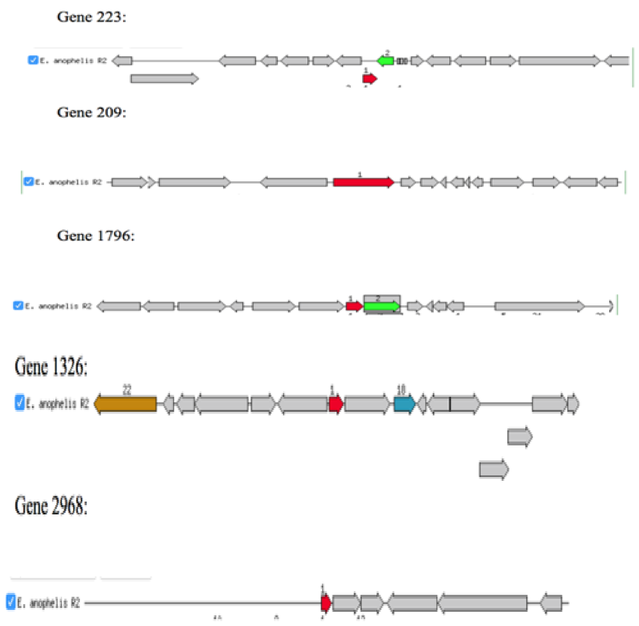
RESULTS

Table 1

Gene Number	Number of Transcriptions Under Control	Number of Transcriptions Under Cefotax	Number of Transcriptions Under Imipenem	Total Number of Transcriptions Under All Three conditions	Fold Change in Transcriptions in Cefotax Compared to Control	Fold Change in Transcriptions in Imipenem compared to Control
1326	2516	3738	2252	8506	1.5	-1.1
209	6	5	7	18	-1.2	1.2
223	1365	389	2118	3872	-3.5	1.6
2968	2	1	5	8	-2.0	2.5
1796	658	1255	402	2315	1.9	-1.6

Table 1 shows level of transcripts for each gene under the three different conditions.

Figure 1



Above are the maps of the location of the five genes within the *E. anophelis* chromosome.

DISCUSSION

Because heat shock proteins are used to help repair damage done to the cell, they are especially important targets for antibiotics. The more times that the gene is present is in the control, the more likely it is that that particular gene is crucial to the survival of the bacteria.

Out of the five genes we looked at, gene 1326 was the most common in the control and was transcribed 2516 times. This number went up significantly, 1,222 more times, when *E. anophelis* was exposed to Cefotax. Conversely, this number went down slightly under Imipenem, but not enough to be considered a significant change. Only Cefotax significantly altered the transcription levels.

Gene 223 appeared a total of 1,365 times in the control. The number of transcriptions decreased dramatically when *E. anophelis* was exposed to Cefotax. The gene was transcribed 976 less times in the Cefotax than when in the control. However, the number of transcriptions increased significantly under Imipenem, and gene 223 was transcribed 753 times more. These numbers show that both Cefotax and Imipenem significantly changed the transcript levels.

Gene 1796 was the third most common gene in the control and was transcribed 658 times. When exposed to Cefotax, the gene transcribed 597 more times than it did under the control. On the flip side, the number of transcriptions within the Imipenem decreased by 256 times. Therefore, both Cefotax and Imipenem significantly altered the transcription levels.

Gene 209 appeared only 6 times under the control. When in Cefotax, the change in transcriptions was not significant. It was not significant when exposed to Imipenem, either. This shows that neither antibiotic significantly altered the level of transcripts.

The gene that was present the least was 2968. This gene appeared two times in the control, an incredibly low number given the size of the genome of *E. Anophelis*. The gene was transcribed once in the Cefotax, a decrease from the number of times the gene transcribed under the control. Five transcriptions occurred when the gene was exposed to Imipenem, which was an increase of 3 from the number of transcripts in the control. Therefore, both antibiotics significantly changed the transcript levels.

We did see significant change in gene 2968. However, the number of transcripts were negligible, and therefore could be reasoning for false identification as a significant change.

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