Testing for the Expression of Different Genes in the Presence of Antibiotics in Elizabethkingia Anophelis

GRP# 20

Austin Linthacum, Ty Hess, Jasmin Garcia, Nathanial Torres, William Johnson, Dr. Patricia Canaan

ABSTRACT

As a group we found and briefly defined a pathway of genes found in Elizabethkingia anophelis R26. The pathway of genes we found were under the Cobalt-Zinc-Cadmium Resistance. In addition to defining these genes we also found their DNA sequences. After having this information we decided to then look at each individual gene's RNA sequence and we recorded the transcription pattern for each of the genes in each the conditions given. The three antibiotics or conditions given were Cefotax, Imipenem, and control. After analyzing the information we just collected, we had to decide what these significant increase or decrease in numbers, depending on the condition given, meant.

INTRODUCTION

We have researched genes inside the bacteria Elizabethkingia Anophelis. Elizabethkingia Anophelis is a deadly bacteria that recently had an outbreak in Wisconsin. In November 2015 in Wisconsin 48 people were diagnosed as infected with at least 18 resulting in death. Elizabethkingia Anophelis is exceptionally resistant to antibiotics so it would be very beneficial to determine what helps this pathogen able to resist these antibiotics so well.

The genes CusR, CzcA, CzcC, CusB, and TR are Cobalt Zinc Cadmium genes that help with antibiotic resistance. Resistant determinant genes are usually located on a plasmid. TR is a transcriptional regulator of the MerR family which is a group of metal responsive regulators within the genome. Genes 369, 370, 996, 999 and1471 are all a part of a large family of genes that are crucial to the bacteria's ability to defend against antibiotics. We specifically focused our tests on these five particular genes and proceeded to test them against two antibiotics, Cefotax and Imipenem, along with a control sample.

Our goal is to analyze and better understand these genes and then record the effects that different antibiotics have on them, therefore we will be looking specifically at the number of mRNA counts that are expressed when the genes are in the presence of the two antibiotics. We believe that these genes will show some response to at least one of the antibiotics that they are to be tested against them in this trial.

MATERIALS AND METHODS

- Electronic Device with Access to Online References

1. After navigating to the Rast Database, the genes coding for cobalt-zinc-cadmium resistance were located through the search engine and the first five (with the exception of genes coding for hypothetical or probable proteins) were identified then described with outside sources like Wikipedia.
2. Using the Rast Database, the DNA sequence for each gene was copied and pasted into the Blast Database to determine the contigs of imipenum, cephalo, and the control. After determining whether each gene was expressed under those circumstances, data pertaining to the alignment scores was recorded along with any significant differences.
3. Using the chart for the RNA sequence data of fold changes for E. anophelis R26, the fold changes for each gene were recorded with an emphasis on significant changes (an increase or decrease by at least 50%).
4. After searching for each gene in the Rast Database, the visual region diagram was located and copied into the results so that the surrounding genes could be seen and analyzed for any alignment patterns.

RESULTS

Genes Associated With Cobalt-Zinc-Cadmium Resistance:

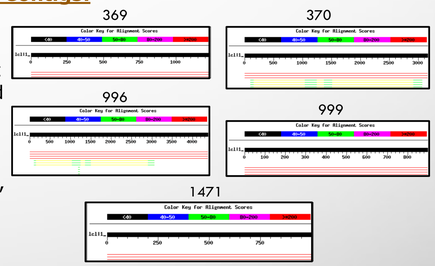
Gene ID	Function
369	Cobalt-zinc-cadmium resistance CzcA; cation efflux system protein CusA
370	Cobalt-zinc-cadmium resistance CzcA; Cation efflux system protein CusA
996	Cobalt-zinc-cadmium resistance CzcA; Cation efflux system protein CusA
999	Cobalt-zinc-cadmium resistance CzcD
1471	Cobalt-zinc-cadmium resistance

Each gene coded for the cobalt-zinc-cadmium resistance protein (Czc) with the exception of genes 369, 370, and 996 which also coded for a cation efflux system protein (CusA) as well. While genes 369, 370, and 996 all contained CzcA, gene 999 contained CzcD in contrast.

The main function of Czc is to detoxify the periplasm by export of toxic metal cations from the periplasm to the outside. The efflux systems located within genes 369, 370, and 996 function to detoxify the cytoplasm.

RNA Transcript Contigs:

All of the genes hit contigs in all three of the libraries for imipenum, cephalo, and the control when their DNA sequences were input into the blast search engine meaning that they were all expressed under these conditions. Each alignment score also contained at least three red lines showing a high distribution on the query sequence. Genes 370 and 996 also hit contigs for a second value of imipenum, cephalo, and the control that had green and yellow lines within the alignment score meaning that there was a lower distribution on the query sequence.

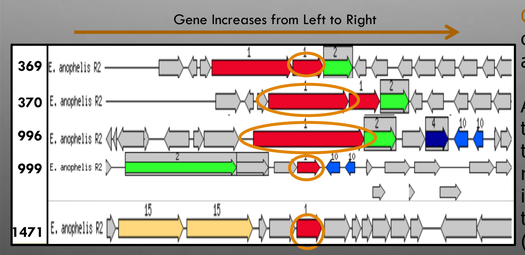


Transcription Patterns and Surrounding Genes:

Gene	Cefotax Fold Change	Imipenem Fold Change	Total mRNA	mRNA Control	mRNA Cefotax	mRNA Imipenem
369	-1.1	-1.3	1316	481	456	379
370	-1.1	-1.2	1672	610	547	515
996	-1.5	1.1	407	146	100	161
999	-1.4	1.4	84	27	20	37
1471	-3.8	1.8	3389	1117	297	1975

Most of the transcription patterns showed insignificant changes compared to the control. Overall, there were only three significant fold changes. For gene 996, there was a -1.5 fold change for Cefotax. For gene 1471, there was a -3.8 fold change for Cefotax and a 1.8 fold change for Imipenem. Each gene had a relatively high level of abundance ranging from the 400s to the 3000 except for gene 999 which had a total mRNA count of 84. Gene 1471 was the only one with a transcription score reaching the thousands. All of the fold changes decreased when the antibiotic Cefotax was used and all of the fold changes increased when Imipenem was added except for genes 369 and 370.

After examining the surrounding genes of the cobalt-zinc-cadmium genes, we found that most of them had proteins similar or related to the function of the Czc genes we identified. They had different functions, but they were genes related to it in some way (i.e. cation efflux system of metals).



DISCUSSION

We found a pathway of genes found in Elizabethkingia anopheles R26 and how these genes reacted when put into different conditions. We collected information from three different conditions. The three conditions we used were control, Cefotax, and Imipenem. Out of our five genes three of them were not effected by either condition enough to be a significant change. The genes that did not have a significant change were gene numbers 369, 370, and 999. However one of the genes we choose had a significant change when given Cefotax but had little effect when given Imipenem. This gene was gene number 996. Our last gene, gene number 1471 was significantly effected by both Cefotax and Imipennem.

The reason for this study was to find out what genes in Elizabethkingia were significantly effected under each condition, so we could try and figure out a way to treat this bacteria. We believe that two of our genes are required for the survival of Elizabethkingia. These two genes are gene number 996 and 1471. We also hypothesized that our other three genes are not required for the survival of the bacteria.

REFERENCES

• Moore, L 2013 "Elizabethkingia Anophelis - MicrobeWiki." Wikipedia. 1. 1 https://microbewiki.kenyon.edu/index.php/Elizabethkingia_anophelis.
• Aziz et al., [http://www.ncbi.nlm.nih.gov/pubmed/18261238?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum| BMC Genomics], 2008 ([http://www.theseed.org/RASTPaperSupplementalMaterial/index.html)
•Altschul, Stephen F. (1991). Amino acid substitution matrices from an information theoretic perspective. J. Mol. Biol. 219:555-65
•Altschul, S. F. (1993). A protein alignment scoring system sensitive at all evolutionary distances. J. Mol. Evol. 36:290-300.