C5-branched Dibasic Acid Metabolism: Does it Matter?
An Investigation into the Expression of the C5-Branched Dibasic Acid Metabolism Pathway

GRP# 9

George Crane, Kidist Beker, Brianna Willard, and Dr. Patricia Canaan

ABSTRACT

Elizabethkingia anopheles is resistant to majority of antibiotics making it difficult to fight the infection. The goal of the experiment was to research a genome and tell if it is transcribed in the bacteria. After sequencing the DNA of the gene in Patric database, the BLAST system was used to convert the sequence to the RAST sequence. The spread sheet R26 RNA sequence data was used to determine if the amount of expression was significant. All of the genes were significantly expressed except the acetoacetate synthase small subunit.

INTRODUCTION

Elizabethkingia anopheles is a strain of the Elizabethkingia species of bacteria originally found in mosquitos. It has the potential to cause human disease which, in some cases, can lead to death. So far, the largest outbreak of Elizabethkingia has been centralized in Wisconsin, although cases have also been documented in Michigan. Currently, *Elizabethkingia anopheles*, is resistant to over twenty types of antibiotics making an infection nearly impossible to cure. The overall goal of the experiment is to research a specific pathway in the R26 genome to see if the genes are transcribed in variants of *Elizabethkingia anopheles* grown with Cefotaxime and Imipenem. The chosen pathway is a C5-branched dibasic acid metabolism pathway. Using the RAST data base and data provided by Dr. Canaan, it will be possible to tell whether or not the five genes within this pathway are actively expressed in the variant strains of *Elizabethkingia anopheles.*

MATERIALS AND METHODS

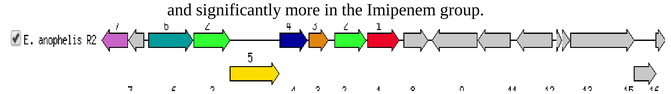
First the pathway, C5-branched dibasic acid metabolism, was chosen at random from the Patric database. Researching the C5-branch dibasic acid metabolism pathway there are five different genes: succinyl-CoA ligase beta chain, succinyl-CoA ligase alpha chain, alpha-acetolactate, acetolactate synthase small subunit, and acetolactate synthase large subunit. Then obtain the DNA sequence from the Patric database for each of the genes. Then convert the Patric sequence to the RAST sequence. In order to obtain the RAST sequence, the data form the PATRIC data base was run through a BLAST system. Using the RAST sequence data and the R26 RNA sequence data spread sheet, it was determined that all of the genes in our pathway were significantly expressed expect for the acetoacetate synthase small subunit. In order to determine if the gene was transcribed or not, the fold change between the control group and the variant groups had to be greater than fifty percent.

RESULTS

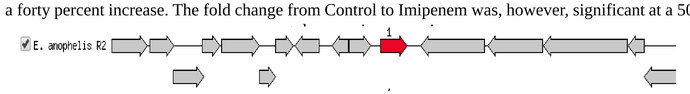
RAST Name	Fold Change: Cefotax/Control	Fold Change: Imipenem/Control	Total mRNA	Control mRNA	Cefotax mRNA	Imipenem mRNA
Peg.292	-3.8	2.5	3505	929	245	2331
Peg.1900	1.4	-1.5	21784	7027	10071	4686
Peg.2410	-1.8	1.1	48	18	10	20
Peg.2586	-1.3	-1.1	360	136	105	119
Peg.2587	-1.6	-1.2	44	18	11	15

Through the use of the Patric data base, the RAST data base, and a BLAST system, the selected genes were able to be seen in the RNA sequencing data provided by Dr. Canaan. It is clear in the data that each of the five genes, within the C5-branched dibasic acid metabolism pathway, are all expressed to a varying degree. This includes the control, the sample treated with Cefotaxime, and the sample treated with Imipenem.

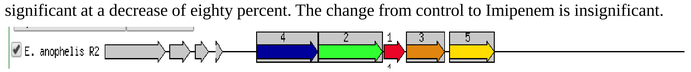
Gene Peg. 292 is expressed to a considerable extend in all three samples, but has the most significant showing in the Imipenem group. A significant difference of expression within samples is fifty percent or greater. As seen in the chart above, gene 292 is expressed significantly less in the Cefotaxime group, and significantly more in the Imipenem group.



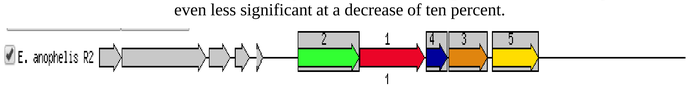
Gene Peg. 1900 is the most expressed gene within the C5-branched dibasic acid metabolism pathway. The most significant expression is within the Cefotaxime variant at 10071 hits. Although it is the most expressed, the fold change from control group to the Cefotax group is not a significant change at only a forty percent increase. The fold change from Control to Imipenem was, however, significant at a 50



Gene Peg. 2410 yields a large decrease in expression compared to the previous two genes. Gene 2410 is the least expressed gene in the pathway; however, the fold change from control to Cefotaxime is significant at a decrease of eighty percent. The change from control to Imipenem is insignificant.



Gene Peg. 2586 is expressed at a slightly significant rate with 360 total hits. This, however, does not translate to significant change in expression between samples. The fold change from control to Cefotaxime was a small decrease of thirty percent, while the change from control to Imipenem was even less significant at a decrease of ten percent.



Gene Peg. 2587 is weakly expressed at only 44 total hits. The fold change from control to Imipenem is also insignificant at a decrease of only twenty percent. The fold change from control to Cefotaxime, however, is a significant change at a decrease of sixty percent.

DISCUSSION

The five genes mainly catalyze the breakdown of amino acids or chemical reactions involving mainly ATP and ADP. The gene that is transcribed the most is Succinyl-CoA ligase [ADP-forming] alpha chain. The reason the Succinyl-CoA ligase [ADP-forming] chains are the most transcribed is because it is an enzyme that catalyzes the chemical reaction ATP + succinate + CoA ⇋ ADP + phosphate + succinyl-CoA. Since this enzyme is taking part in a reaction that involves ATP and ADP, it is vital to providing energy in the *Elizabethkinga anophelis* bacteria cells (1,2,3). The genes that are the least transcribed Acetolactate synthase subunits. The function of these genes are to "catalyze the first step in the synthesis of the branched-chain amino acids (valine, leucine, and isoleucine)" (4). The acetolactate synthase enzymes are not transcribed as often as the other enzymes because the breakdown of branched-chain amino acids is probably not as important to the bacteria strain as the others. More transcription means more RNA produced, which in turn leads to the gene being transcribed is more important than the other genes in the pathway. The more important the gene is, there will be more RNA present to be transcribed which leads to more proteins being produced to keep the bacteria functioning properly. For Succinyl-CoA ligase beta chain, there was significant fold changes and very high mRNA counts. This further proves the significance the Succinyl-CoA genes in *Elizabethkingia anophelis*. The Acetolactate synthase enzymes, specifically the small subunit, had no significant fold changes or high mRNA counts. Since the acetolactate synthase enzymes are not as significant as the Succinyl-CoA ligase enzymes, it makes sense that they do not show significant fold change or high mRNA counts. During our research, we discovered specific genes being expressed and learned they are necessary, especially the significant Succinyl-CoA [ADP-forming] ligase. If we can eliminate the important genes that are essential for the survival of this bacteria strain, this could lead to a possible vaccine for *Elizabthkingia anophelis.*

REFERENCES

1. Fraser, M.E.; James, M. N. G.; Bridger, W. A.; Wolodko, W. T. (1999) "A detailed structural description of Escherichia coli Succinyl-CoA synthetase1". *Journal of Molecular Biology.* 285 (4): 1633-1653.
2. https://en.wikipedia.org/wiki/Succinate%E2%80%94CoA_ligase_(ADP-forming)
Kaufman S; Alivasatos SGA (1955). "Purification and properties of the phosphorylating enzyme from spinach". *J. Biol. Chem.* 216: 141-152.
3. https://en.wikipedia.org/wiki/Acetolactate_synthase