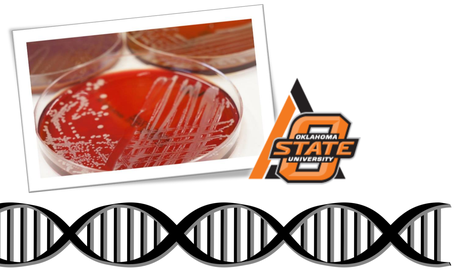
GRP# 12

Our Little Piece of Elizabethkingia

Naima Afrin. Haley Riley. Madison Clark, and Zomaree McNac
Dr. Patricia Canaan. William Johnson, and Nathan Torres



ABSTRACT

Elizabethkingia anophelis is the cause of many disease outbreaks in the United States which makes it a crucial bacteria to research and investigate. Our study focuses on whether certain antibiotics have a significant effect on the RNA transcription levels in five genes involved in the Shikimate pathway. Using the UniPROTdb, RASTdb, and BLAST databases, along with RNA sequencing we were able to find data to aid in our investigation. Ultimately, we found that exposing bacterium to these specific antibiotics leads to significant changes in the transcription levels in two of the five genes that we studied.

INTRODUCTION

Elizabethkingia anophelis is a gram negative human pathogen that is resistant to most antibiotics (10). Bacteria such as *E. anophelis* is known to respond to changes in environment often through gene expression (9). In correlation to that, five genes in *E. anophelis* were subjected to exposure to antibiotics such as Cefotax and Imipenem to research whether a change in environment could lead to a change in the transcription levels of these genes. The results indicated that the antibiotics do indeed have an effect on the RNA transcription levels of the five genes we have chosen.

The Shikimate Pathway

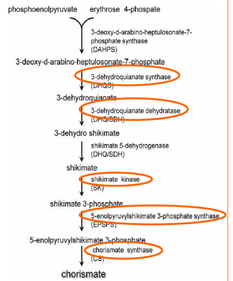


Figure 1. Illustrates the Shikimate Pathway and the enzymes that are required for its functionality. The red circles indicate the enzymes that have already been identified by the UniProtKB database.

MATERIALS AND METHODS

Starting with the UniPRO database, search through the genes (2).

- Click on the number beside a metabolic pathway name to open a page and table with those genes listed.
- Click the (+) symbol to expand each pathway name and scroll down to sub pathways.
- Explore additional Views by clicking on the "Gene Ontology" or "Enzyme Class" in the menu on the left.

After navigating through the UniPRO database, use BLAST to convert the genes to the RAST database (1, 3).
Use the RAST database to obtain details of each specific gene (1).

- Log into the RAST database
- Find *Elizabethkingia anophelis* R26 at the top of the table
- Click "view details" on the right side of the table
- Click browse annotated genome in "seed viewer"
- Click "here" on the right side of the little box
- At the top of the table change the #15 for "display 15 items per page" to 3782 to view all of the predicted genes.

Research the genes and their function using online sources.
Look for corresponding genes in the RNA sequencing data.

RESULTS

We identified five genes related to the Shikimate pathway.

- **3-dehydroquinate dehydratase II** (RAST ID: 1424)
- **Shikimate kinase I** (RAST ID: 222)
- **Chorismate synthase** (RAST ID: 1591)
- **3-dehydroquinate synthase** (RAST ID: 3399)
- **5-Enolpyruvylshikimate-3-phosphate synthase** (RAST ID: 1737)

TRANSCRIPTION LEVELS

RAST Name	Description	Fold Change* (Cefotax/ Control)	Fold Change* (Imipenem/ control)	TOTAL mRNA ** from all 3 conditions	mRNA Counts Control	mRNA Counts Cefotax	mRNA Counts Imipenem
fig|1246994.5.peg.1424	**3-dehydroquinate dehydratase II**	1.5	-2.0	382	127	191	64
fig|1246994.5.peg.222	**Shikimate kinase I**	-5.5	2.2	2582	756	138	1688
fig|1246994.5.peg.1591	**Chorismate synthase**	1.5	1.5	43	11	16	16
fig|1246994.5.peg.3399	**3-dehydroquinate synthase**	1.4	1.6	20	5	7	8
fig|1246994.5.peg.1737	**5-Enolpyruvylshikimate-3-phosphate synthase**	1.3	-1.4	222	75	95	52

Key
- Significant Increase
- Significant Decrease

Table 1. Illustrates the transcription levels of the genes when exposed to Cefotax, Imipenem, as well as, the control group.

GENE LOCATION

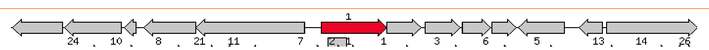


Figure 2. Represents the gene **5-Enolpyruvylshikimate-3-phosphate synthase** and the genes that surround it. An oxidoreductase, short chain dehydrogenase/reductase family is to the left and a NADH dehydrogenase subunit 2 is to the right. (1)

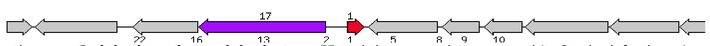


Figure 3. represents the gene **3-dehydroquinate dehydratase II** and the genes that surround it. On the left, there is a putative outer membrane protein that is probably involved in nutrient binding and on the right is Phosphoenolpyruvate carboxykinase (1).

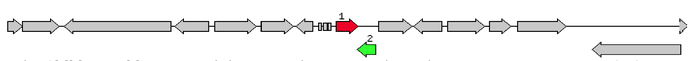


Figure 4. represents the **Shikimate kinase I** and the genes that surround it with tRNA-Lys-TTT, tRNA-Leu-GAG, tRNA-Lys-TTT, on the left and a hypothetical protein on the right (1).

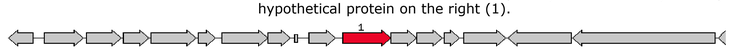


Figure 5. represents the **Chorismate synthase** and the genes that surround it. On the left, is a hypothetical protein and on the right is Thioredoxin (1).

Figure 6. represents the **3-dehydroquinate synthase** and the genes that surround it. A Carbapenem antibiotics biosynthesis protein card was on the left and on the right, is a hypothetical protein (1).

DISCUSSION

- Based on our results, we have concluded that our genes do, in fact, respond to their environment. Two of our five genes experienced a significant change in transcription when exposed to either Cefotax or Imipenem.
  - **3-dehydroquinate dehydratase II**, when exposed to Imipenem, decreased by 50 %. When it was exposed to Cefotax, there was a 50% increase.
  - **Shikimate kinase I** experienced a 80% decrease when exposed to Cefotax, and a 220% increase when exposed to Imipenem.
- We believe that these significant changes represent the organism's attempt to adapt when its survival is put at risk.
- When we examined the genes surrounding our genes of interest, we found that they were unrelated in function. This means our genes are not operons, and they do not rely on each other, instead they function independently.

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