The Presence of Protection from Reactive Oxygen Species Proteins in *Elizabethkingia anophelis* in antibiotics Cefotax and Imipenem

GRP# 29

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INTRODUCTION

Elizabethkingia anophelis, a gram-negative bacterium, has caused a mainly bloodstream infection in 61 people in the states of Wisconsin, Illinois, and Michigan (2,5). This rod-shaped bacterium has led to the deaths of twenty people, all of which had previously underlying illnesses as well (2,5). *E. anophelis* has proven to be resistant to most antibiotics, making treatment difficult (5). Researchers are conducting research on five different genes, 946, 947, 970, 1488, and 1489, which all are in the oxidative stress pathway.

Gene 946 codes for the organic hydroperoxide resistance transcriptional regulator, where it functions in the transcription for the detoxification of organic hydroperoxides (1).
Gene 947 codes for the organic hydroperoxide resistance protein, where it functions in the detoxification of organic hydroperoxides (1).
Gene 970 codes for the protein catalase, which decomposes hydrogen peroxide molecules (3). It also codes for the protein peroxidase, which is thought to be a hemoprotein catalyzing the oxidation by hydrogen peroxide (4).
Gene 1488 codes for the protein hydrogen peroxide gene hydrogen peroxide-inducible genes activator, which is thought to be used to bind DNA.
Gene 1489 codes for the protein catalase, which, like in gene 970, decomposes hydrogen peroxide molecules (3).

ABSTRACT

Not much is known about the bacteria *Elizabethkingia anophelis,* and this project focuses on finding a pathway of genes and seeing if these genes are transcribed within the cell for it to use. The project involves looking at mRNA sequences for the genes that are involved in the Reactive Oxygen Species subcategory of the Oxidative Stress category of *E. anophelis.* The first phase of the research looked at finding the DNA sequences for each gene and finding if the sequences are found in any of three environments: the control, Cefotax, and Imipenem (antibiotics). Then, the second phase was looking at the map of the genome to see where the genes were located and what the surrounding genes were. The last phase was finding exactly how many copies of mRNA were found in the E. anophelis cells when it was submerged into each of the three different environments that included the two antibiotics.

MATERIALS

- RAST Database
- BLAST Database
- RNAseq Data table
- Scientific journals (specified in the references)

METHODS

The researchers used the RAST database to find the subsystems of oxidative stress. They found the RNA sequences for *Elizabethkingia anophelis R26.* Afterwards, the electronic scientific journals showed what the specific proteins did in other gram negative cells. The BLAST database was very helpful in converting the RNA sequences to DNA sequences. Also on BLAST, the researchers found out if the sequence was present in *Elizabethkingia anophelis R26* when in the three environments: control, Ceftotax, and Imipenem. Next, the researchers used a RNA sequence data table to see the increase and decrease in transcription of the specific genes that we are looking at (946, 947, 970, 1488, 1489). After they found the data of the RNA sequences, they saw how many times exactly it was transcribed in each environment and if the change was notable and significant.

RESULTS

Figures 1.1-1.5 represent the BLAST database results which show if the DNA sequence was present in the three environments: control, Cefotax, and Imoipenem.

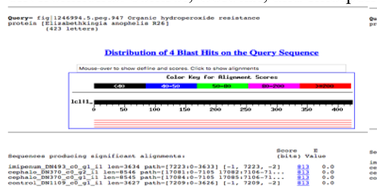

Figure 1.1: Gene 946

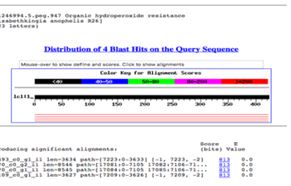

Figure 1.2: Gene 947

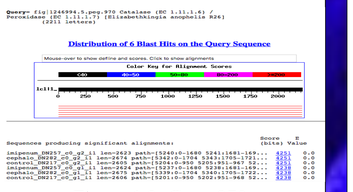

Figure 1.3: Gene 970

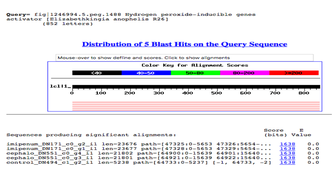

Figure 1.4: Gene 1488

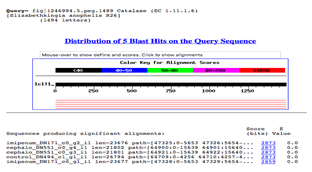

Figure 1.5: Gene 1489

Location of Genes within the Genome

Figure 2.1: Gene 946

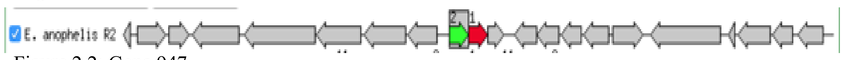

Figure 2.2: Gene 947

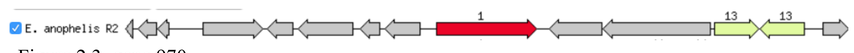

Figure 2.3: gene 970

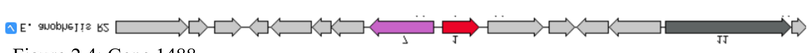

Figure 2.4: Gene 1488

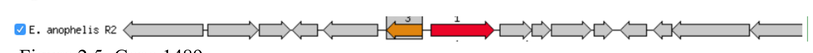

Figure 2.5: Gene 1489

The Percent Increase/Decrease in RNA Production of Genes in Different Environments

Gene	Fold Change (cefotax/control)	Fold Change (Imipenem/ Control)	Total mRNA	mRNA counts control	mRNA counts Cefotax	mRNA counts Imipenem
946 (OhrR)	-1.8	2.0	32	9	5	18
947(Ohr)	-1.6	1.5	35	11	7	17
970	1.3	.1.9	3093	1092	1428	573
1488	1.6	-1.3	1016	302	483	231
1489	1.5	-1.5	1097	348	521	228

Figure 3.1: Data table that shows the number of RNA sequences of each gene present in different environments

DISCUSSION

The pictures in Figure 1.1-1.5 show the results from the BLAST Database when the DNA sequence of the genes were inserted and the results showed if the corresponding RNA sequences were present in the E. anophelis cell when it was in the different environments. From Figure 1.1-1.5 it can be seen that the sequences were present in all three environments (the control and the two antibiotics: Cefotax and Imipenem.

In Figures 2.1-2.5 we have the map of the genome that includes the gene in question. On each map the red arrow is the selected gene. For 946 and 947 we can see that the genes are right next to each other because 946 is a transcriptional regulator (OhrR) for 947 (Ohr). 970 is isolated by itself. To the left of 970 are multiple hypothetical proteins while on the right there are membrane proteins and transcriptional regulators. 1488 and 1489 are right next to each other, which is reasonable as it is thought that 1488 is a regulator for gene 1489. 4 genes to the left of 1488 is gene 1492, which is a protein called Integrase. This is interesting because Integrase is a protein necessary for bacteria to either accept genes that aren't theirs or is used to help transfer copies of their genes to other bacteria. This integrase near the area of 1488 and 1489 indicates that these genes, or other genes in this area, could originally be from another bacterial cell of the same species or a different species entirely, or they can be genes that *E. anophelis* readily copies and transfers to other cells to help give them immunity to different types of antibiotics.

In Figure 3.1 the data table shows the percent increase or decrease in the production of each gene's RNA sequence when *E. anophelis* is subjected to different environments. Anything that is above a 1.5 represents a 50% increase increase or more and anything below -1.5 represents 50% decrease in production or more. From the data we know that both 946 and 947 decreased in Cefotax and increased in imipenem. As shown from Figures 2.1 and 2.2, we know that 946 and 947 are next to each other and 946 controls the transcription of 947. This explains why the RNA sequence production mirrors each other in the environments. From the significant decrease it can be concluded that genes 946 & 947 aren't necessary for the cell to have when exposed to Cefotax, but is necessary to help protect the cell from Imipenem as seen by the significant increase in RNA production of both genes. Gene 970 is a thought to be both a catalase and peroxidase protein. From the information in the RNA sequence data table we can tell that this gene has a big function within the cell because of the large amount of repeated copies of RNA for that gene found in the cell. There was a slightly less than 50% increase in production of 970's RNA when the cell was exposed to Cefotax and a greater than 50% decrease in production when exposed to Imipenem. This shows that the protein it produces is not absolutely necessary for *E. anophelis* to have when exposed to Imipenem because a little over 1/2 of the original amount of RNA is produced. The increase in RNA when in Cefotax shows that the protein is helpful and used by the cell. Genes 1488 and 1489 are closely associated as they are located right next to each other in the genome and it is believed that 1488 regulates 1489. 1488 is a Hydrogen peroxide-inducible genes activator and 1489 is a catalase of sorts. The reason it is thought that these two genes go hand in hand is because in the RNA sequence data table the two have almost identical percentage increase and decreases for both Cefotax and Imipenem, and the amount of mRNA found in the cell for each environment is almost identical as well. This data further shows that since there is a increase in mRNA for 1488 and 1489 when *E. anophelis* is in Cefotax, the proteins must be necessary for the cell to use as a defense mechanism against Reactive Species of Oxidative Stress. Meanwhile the decrease in mRNA for both genes when E. anophelis is in Imipenem shows that the proteins are still needed by not necessary for the cell to have when dealing with this antibiotic.

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