Penicillin and Cephalosporin Biosynthesis

GRP# 33

Abbi Wright, Alex Partida, and Austin Moore

ABSTRACT

Elizabethkingia anophelis is a bacteria that causes human disease (1). It is most commonly found in the gut of mosquitoes. Characteristics include that the bacteria are rod-shaped, arranged singularly or in pairs, and appear Gram Negative (1).The Elizabethkingia genome is made of many pathways. Using various databases provided to us, we were able to isolate a pathway called Penicillin and Cephalosporin Biosynthesis, which is made up of 5 beta-lactamase genes. Beta-lactamase are enzymes that provide resistance to antibiotics such as penicillin, cephamycin, and carbapenems (2). All five of these genes were expressed in all three conditions, meaning that it hit contains in all three libraries, but only one gene expressed significant fold changes. Each of our were each resistant to penicillin. This led us to the conclusion that the Elixabethkingia bacteria as a whole is very resistant to penicillin, which is highly unusual for there to be multiple genes that are this resistant.

INTRODUCTION

Strains of bacteria like Elizabthkingia are resistant to antibiotics through the production of beta-lactamases (1,2). In order to understand a specific antibiotic that Elizabethkingia is resistant to, we chose to focus on the five genes in our pathway that are identified as betal-lactamase. These genes are resistant through a process called hydrolysis of the beta-lactam ring, as shown in figure 1 (3).

MATERIALS AND METHODS

To obtain the gene sequences for our 5 genes in the Penicillin and cephalosporin biosynthesis pathway, we used the PATRIC database (4). We inserted these gene sequences into the BLAST database (5) to find the corresponding genes in the RAST database (6). We then used the FASTA sequences from RAST to obtain BLAST results for each gene in our pathway. Our BLAST results gave us showed us which of our genes were expressed in each condition.

RESULTS

From the fold changes spreadsheet provided to us, we discovered that only one of our five genes were expressed, or showed signs of significant change. We also found that all five of our genes were expressed in all three conditions, as shown in the Distribution of Blast Hits for one of our beta-lactamase genes (Figure Two). We also learned that our 5 beta-lactamase genes are very resistant to penicillin. They disable molecules of penicillin and resist its effects as shown in Figure One, a diagram of a beta-lactam ring.

Figure 1

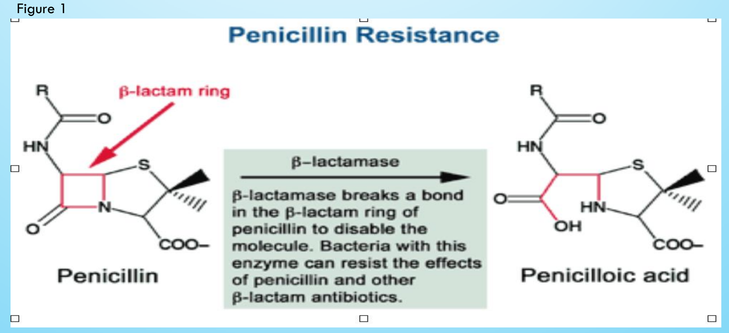

Figure 2

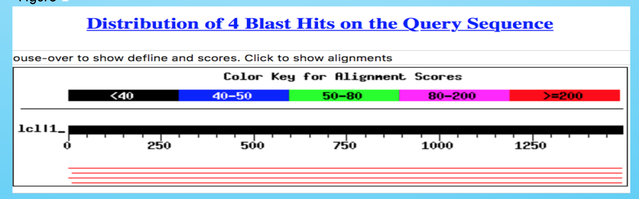

Figure 3

Genes from Penicillin pathway	Control	Cefotax	Imipenem	Total Transcripts Observed	Fold Change in Cefotax compared to Control	Fold Change in Imipenem compared to Control
Gene 1 Beta-lactamase	24	19	21	64	-1.3	-1.1
Gene 2 Beta-lactamase	508	600	354	1462	1.2	-1.4
Gene 3 Beta-lactamase	71	56	32	159	-1.3	-2.2
Gene 4 Beta-lactamase	48	19	37	104	-1.5	-1.3
Gene 5 Beta-lactamase	116	132	109	357	1.1	-1.1

DISCUSSION

Our results show that only Beta-lactamase gene 3 was expressed, as shown in Figure 3. Beta-lactamase gene 3 has a fold change in Imipenem compared to its control of 2.2. Its control is less than -1.5, which makes it significant. This gene is down regulated, meaning that it produces less of its specific gene. Possible explanations for this could be that the pathway doesn't need any more of that specific gene, or there could even be a negative effect on the pathway if it were creating more of that gene. Compared to some other genes in the spreadsheet, there is little variation in our genes. The rest of the genes in our table did not have fold change numbers that were less than or equal to -1.5 or greater than or equal to 1.5, which is why they are categorized as insignificant. Our total mRNA for each gene was higher than many of the surrounding genes on the spreadsheet.

REFERENCES

1. https://en.wikipedia.org/wiki/Elizabethkingia_anopheles

2. https://en.wikipedia.org/wiki/Beta-lactamase

3. http://www.wiley.com/college/pratt/0471393878/student/activities/bacterial_drug_resistance/index.html

4. https://www.patricbrc.org/portal/portal/patric/CompPathwayTable?cType=genome&cId=1246994.3&algorithm=PATRIC&ec_number=

5. http://darwin.biochem.okstate.edu/blast/blast1990.html

6. http://rast.nmpdr.org/rast.cgi?page=Jobs