ATP Synthase EXPOSED

GRP# 8

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ABSTRACT

Elizabethkingia anophelis is a multi-drug resistant and pathogenic bacteria. We decided to investigate the Oxidative phosphorylation pathway in the Energy Metabolism pathway class that contains eight unique genes as found in PATRIC database (4). We went in and found this pathway and the group of unique genes and described each individually. We then took each unique gene and found its DNA sequence data and the genome for the surrounding genes in a spreadsheet that was provided to us. Finally, we compared transcription rates of our genes when exposed to cefotax and imipenem in comparison to control samples. We found that exposure to cefotax results in a trend of up-regulation of ATP Synthase gene transcription. Conversely, exposure to imipenem resulted in a trend of downregulation in transcription levels.

INTRODUCTION

E. anophelis is a strain of bacteria found inside mosquitos that has resulted in the death of many in the Wisconsin area. *E. anophelis* has been known for being resistant to many different types of antibiotics, making it difficult to treat (2). As we explored *E. anophelis* we found one protein in particular called ATP Synthase that we decided to study. Whenever a bacteria is paired with an antibiotic the transcriptions levels in the particular genes may change (3). So, when studying the genes in ATP Synthase our main purpose was to look at the transcriptions and whether they were up-regulated or down-regulated when paired with two beta-lactam antibiotics: cefotax and imipenem. We began seeking how many transcripts for ATP Synthase were present and what genes surround our genes of interest. During the process of finding these strains, we discovered that two of the transcripts, the Beta chain and Epsilon chain, were not grouped together with the other six genes, the Alpha Chain, Gamma Chain, Delta Chain, Subunit A, Subunit B, and Subunit C, leading us to look more into the transcriptions to find out why. After finding the this information about the strains of ATP synthase, we used this to look at the transcription levels with a control, Cefotax, and Imipenem to see if they were altered, and then decided if there was a significant change from the fold change, to see if there were any similarities or differences between transcription levels when comparing the control sample to the treated samples.

MATERIALS AND METHODS

Our research began with each member of the group looking at the unique genes of *the E. anophelis* genome. We each had to choose two genes/proteins and described them. We then discussed with each other to figure out what pathway or group of interesting genes to investigate and describe them. We decided to investigate the oxidative phosphorylation pathway which contains eight unique genes responsible for encoding the subunits of ATP synthase. These subunits include the alpha, beta, delta, gamma and epsilon subunits of the F1 sector and the a, b, and c subunits of the F0 sector. We located this pathway and the corresponding genes in the PATRIC database (4). When we first looked at these unique genes we noticed that the epsilon chain was not in the database. We hypothesized that it was just not noticed or was just missing from the database. Initially we excluded the epsilon chain from analysis, however, the gene was later found in the RAST database, and was therefore included (5). We took the genes of interest and found the DNA sequence for each individual gene through a BLAST search (1). We went into the PATRIC database and took the last four numbers from the PATRIC database after the - peg. - and then went into the RAST database. In the RAST database we took the last four numbers of each unique gene and placed them into the search bar to find that specific gene so that we could find the DNA sequence. We found our gene we were looking for and clicked on the sequence tab and received the sequence for that unique gene.. We used the BLAST database to match our sequences of the *E. anophelis* R26 genome. These three databases; RAST, BLAST, and PATRIC were major parts in our research in finding the genes and other different information that was needed. We then moved on to look at the RNA sequence of each individual gene and to see if the genome is flanking genes which may be related. Finally, we used a spreadsheet with information regarding transcription levels under the stress of imipenem and cefotax compared to control samples to analyze whether our genes of interest were up-regulated or down-regulated in response to antibiotic exposure. Fold changes in transcription levels were considered significant if the value achieved was greater than 1.5 or less than -1.5

RESULTS

Exposure to Imipenem and cefotax resulted in a change in transcription levels across multiple genes in the oxidative phosphorylation pathway of *E. anophelis*. The gene encoding the beta subunit of ATP synthase was transcribed in significantly greater numbers (50% increase) when the culture was exposed to cefotax. The same gene was transcribed less when exposed to imipenem, though not by a significant level. For the gene encoding the epsilon subunit, transcription increased by 100% when exposed to cefotax and remained the same when exposed to imipenem. The gamma subunit gene transcription increased by 100% when exposed to cefotax and decreased by 50% when exposed to imipenem. Transcriptions for the alpha subunit did not change by significant values when the culture was exposed to either antibiotic. Transcriptions for the delta subunit did not change when the culture was exposed to cefotax, but interestingly increased by 100% when exposed to imipenem. Transcription levels were not altered significantly by exposure to either antibiotic in respect to the "c" subunit gene. For the" a" subunit, gene transcription increased by 50% when the culture was exposed to cefotax and decreased by 80% when exposed to imipenem. Finally, transcription levels for the "b" subunit gene increased by an insignificant amount when exposed to cefotax and decreased by 60% when exposed to imipenem (Figure 1).

	Control	Cefotax	Imipenem
Beta	13	20 (1.5)	9 (-1.4)
Epsilon	23	47 (2.0)	23 (1.0)
Gamma	120	244 (2.0)	78 (-1.5)
Alpha	126	146 (1.2)	117 (-1.1)
Delta	105	106 (1.0)	232 (2.2)
F0 subunit c	110	125 (1.1)	97 (-1.1)
F0 subunit a	182	281 (1.5)	102 (-1.8)
F0 subunit b	36	47 (1.3)	23 (-1.6)

Figure 1. Comparisons in transcriptions levels of the genes responsible for encoding the subunits of ATP synthase across two stresses: exposure to cefotax and exposure to imipenem. The leftmost values in each column represent actual transcriptions while the rightmost values indicate fold changes in comparison to the control sample. Green highlights indicate significant up-regulation. Red highlights indicate significant down-regulation. Yellow highlights indicate insignificant changes in transcription levels. Values were considered significant if the fold change in comparison to the control sample were greater than or equal to 1.5 or, alternatively, less than or equal to -1.5.

Significant Down-Regulation
Significant Up-Regulation
Insignificant Change

Since our genes of interest produce proteins which ultimately conjugate to form ATP synthase, we hypothesized the genes would be located close to one another on the genome to be perhaps transcribed as an operon. The RAST database allowed us to locate a particular gene of interest and analyze the genes found arbitrarily nearby (5). We discovered six of the eight genes of interest are directly next to one another on the genome. The other two genes are located in a different location on the genome approximately 900 genes away. Specifically, the genes encoding for the alpha, delta, gamma, a, b, and c subunits were adjacent to one another while the genes responsible for producing the beta and epsilon subunits were located elsewhere on the genome (Figure 2).

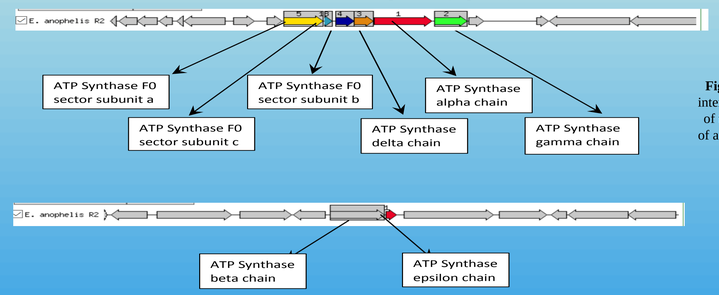
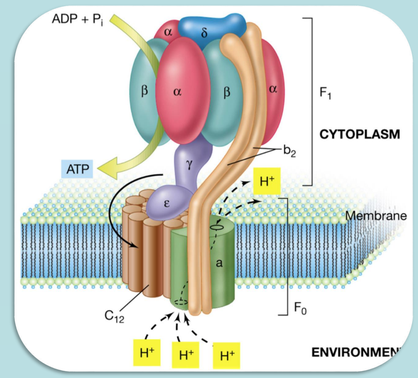


Figure 2. Location of the genes of interest in relation to one another. six of the eight genes appear to be part of an operon while two others appear to be located and transcribed elsewhere on the genome.

Because ATP synthase is highly conserved across all genera, we were able to compare the ATP synthase genes from *E. anophelis* to other bacterial genera. We found that a vast majority of genera share the same location of genes on their genomes with the beta and epsilon chain separate from the remaining six. This could be the result of a difference in time between the evolution of the six genes found adjacent to another and the two found elsewhere on the genome.



Model of bacterial ATP Synthase

DISCUSSION

We looked at multiple genes in the oxidative phosphorylation pathway of *E. anopheles,* more precisely the alpha, beta, delta, gamma, epsilon, a, b, and c subunits, and looked at the changes in their transcription when exposed to cefotax and Imipenem. When exposed to cefotax, which breaks down and ruptures the cell wall killing the bacteria (6), most of the genes have an increased transcription rate which we can hypothesize as an increase in ATP production for enzymes to repair the cell wall and keep the bacteria alive. Further research into this could give us a better explanation to why this happens. When the listed genes are exposed to imipenem, which is bactericidal to rapidly multiplying cells and affects the cell wall inhibiting cell multiplication and growth (7), most of the genes have a decreased transcription rate. With that information we can hypothesize that the cells not being able grow or multiply would decrease energy production thus decreasing the production of ATP. Again, further research into this can better explain the reason this negative transcription happens. The genes that weren't transcribed by much we deemed to be unaffected by the antibiotics and so they didn't have to produce energy. In our research on the RAST database, we found the beta and epsilon genes to be separated from the rest of the genes that were right next to each other in the genome. We think this could be a process of evolution between the separate pieces but it could also be due to an error of the placement in the database. We could use this information to help further the studies of other researchers over the *E. anopheles* bacteria and bring up some ideas to complete the process that we started here.

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