Life in Plastic…It's Fantastic:
Degradation of Bisphenol A in Elizabethkingia Anophelis affected by Cefotax and Imipenem

GRP# 30

Sarah Soliman, Kanako Matsumoto, Christian Wilson, Olivia Davis, Dr. Patricia Canaan, William Johnson, and Nathaniel Torres

ABSTRACT

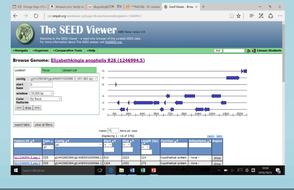
When researching the *Elizabethkingia anophelis* R26 genome, we utilized the RAST database and PATRIC database to find a specific pathway that was important in understanding this bascteria. We used the PATRIC database to find five genes from the Bisphenol A degradation pathway, the same chemical that is commonly found in plastic. BPA is a health concern today for the possible effects it has on our health and brain (1). We each researched a different gene and found what functions they do and what proteins or enzymes they contain. In general, the genes we researched had several similarities, for most of the genes catalyze or reverse certain reactions for a type of alcohol. Also the majority contained the enzyme dehydrogenase, present in four out of the five genes researched (2, 3). After researching each gene and recording all of our data, we went back to the PATRIC database (4) to obtain the DNA sequence of the genes. BLAST allowed us to find our specific genes in the RAST database (5) and view their placement in the E. *anophelis* genome. The gene numbers from RAST were used to find the RNAseq and record the transcription patterns expressed in Cefotax and Imipenem. For some genes, their level of expression increased in the presence of Cefotax and Imipenem, while for others their expression decreased.

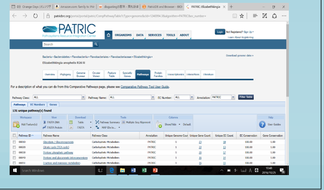
INTRODUCTION

The research we conducted this semester was over the R26 Elizabethkingia anopheles genome. Elizabethkingia anopheles had an outbreak in Wisconsin, Michigan, and Illinois in the spring of 2016. There was around 60 cases of E. anopheles and 18 deaths total in the outbreak. This lead to what we are researching. We selected a group of genes that were all in a similar pathway, Bisphenol A degradation, and researched different aspects. This included the functions of each gene, the RNA sequence of each gene, and their placement in the E. *anophelis* genome.

MATERIALS AND METHODS

We used PATRIC and RAST web pages to examine genes from the Bisphenol A degradation pathway found in the E. *anophelis* bacteria. We researched the five genes associated with this pathway and found their corresponding IDs and nucleotide sequences on the PATRIC database. Originally using PATRIC ID's we used BLAST to convert these PATRIC IDs to RAST IDs. Using RNA sequence data we were able to follow the transcription pattern observed for this Bisphenol A degradation pathway in control conditions, Cefotax, and Imipenem. From this we observed the changing levels of transcription to identify how E. *anophelis* responds to certain chemical environments.





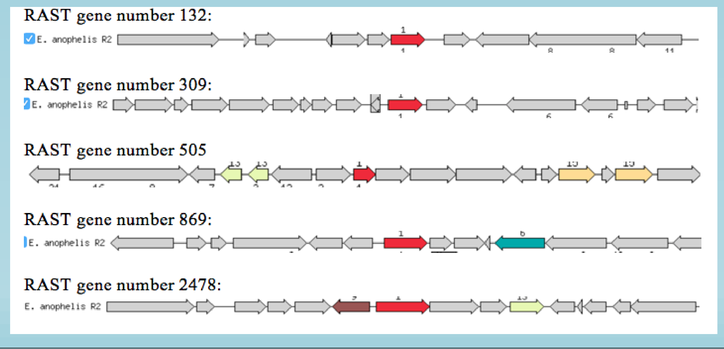
RESULTS

We started out by locating the BPA Degradation Pathway genes in the data spreadsheet containing the levels of RNA transcripts produced by *E. anophelis* grown in Cefotax, Imipenem, and control conditions. We noted any changes in the level of transcription of these genes.

RAST Gene #	Level/# of transcripts observed in *E. anophelis* grown in Control Conditions	Level/# of transcripts observed in *E. anophelis* grown with Cefotax	Level/# of transcripts observedvin *E. anophelis* grown with Imipenem	Total Level/# of transcripts in *E. anophelis* across all three conditions	Fold change in transcription in Cefotax	Fold change in transcription in Imipenem
132	1	1	1	3	-1.0	-1.0
309	432	261	967	1660	-1.7	2.2
505	1	1	1	3	-1.0	-1.0
869	216	166	196	578	-1.3	-1.1
2478	2	1	4	7	-2.0	2.0

RAST Gene number 132 and number 505 show no change in the level of transcripts produced in the presence of Cefotax or Imipenem. There is a 0% increase and 0% decrease in the level of transcripts observed with these two genes in the presence of Cefotax and Imipenem. RAST Gene number 309 shows a significant decrease of expression (more than 50%) when grown in Cefotax as well as a significant increase (more than 50%) when grown in Imipenem. The level of transcription of RAST Gene number 869 decreases slightly when grown with Cefotax and also slightly when grown with Imipenem. However, these levels of decrease are less than 50% when compared to the transcription of Gene number 869 under normal conditions and are thus not considered significant changes. *E. anophelis* R26 also shows a 50% decrease of RAST Gene number 2478 expression when grown with Cefotax and a 100% increase of RAST Gene number 2478 when grown with Imipenem.

Genes that make up the Bisphenol A Degredation Pathway in *E. anophelis* are not on the same chromosome. The five genes are not in the same chromosomal region either.
Images of the genes' placement within the *E. anophelis* genome are shown below. Each gene is indicated as a red arrow.



DISCUSSION

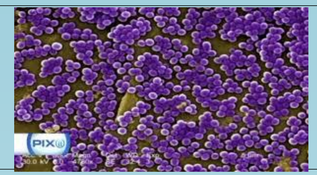
Over the course of the semester we found that Elizabethkingia anophelis contains genes that participate in Bisphenol A degradation. This genes are 2478, 869, 505, and 132 (RAST) were examined in controlled situations: Cefotax and Imipenem. Data allowed us to see that each varied differently, relating to how these cellular functions react in certain situationss.

When Cefotax is present, *E. anophelis* R26 produces a significantly lower amount of bifunctional protein zinc-containing alcohol dehydrogenase and quinone oxidoreductase. It also produces a smaller amount of NADH-dependent butanol dehydrogenase A and multiple polyol-specific dehydrogenase. However, it is difficult to tell if the change in multiple polyol-specific dehydrogenase is due to the Cefotax because it was not highly expressed in *E. anophelis* R26 under normal conditions.

When Imipenem is present, *E. anophelis* R26 produces a significantly larger amount of biofunctional protein zinc-containing alcohol dehydrogenase and multiple polyol-specific dehydrogenase. It also produces a slightly lower amount of NADH-dependent butanol dehydrogenase A.

After obtaining our results, it appears that the Bifunctional protein zinc-containing alcohol dehydrogenase produced from the RAST gene number 132 and the enzyme protoporphyrinogen oxidase IX produced from the RAST gene number 505 may be involved in the normal everyday functions of the cell because the levels of their transcription did not change from the control conditions to the experimental conditions. However, the transcription of the Bifunctional protein zinc-containing alcohol dehydrogenase produced from the RAST gene number 309 changed significantly in the presence of Imipenem and Cefotax, suggesting that these alcohol dehydrogenase proteins are used for a function other than normal cellular functions.

Genes that make up Bisphenol A degradation are not of similar nature, neither of the five genes are even in the same region of the chromosome. Each gene, however, is consistently coupled with at least four other species on the chrimosome. RAST provided great diagrams to better understand the location of these genes. Our research provided information on presence of Impipenem in Bisphenol A Degradation, a viable pathway in E. *anophelis.*



REFERENCES

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