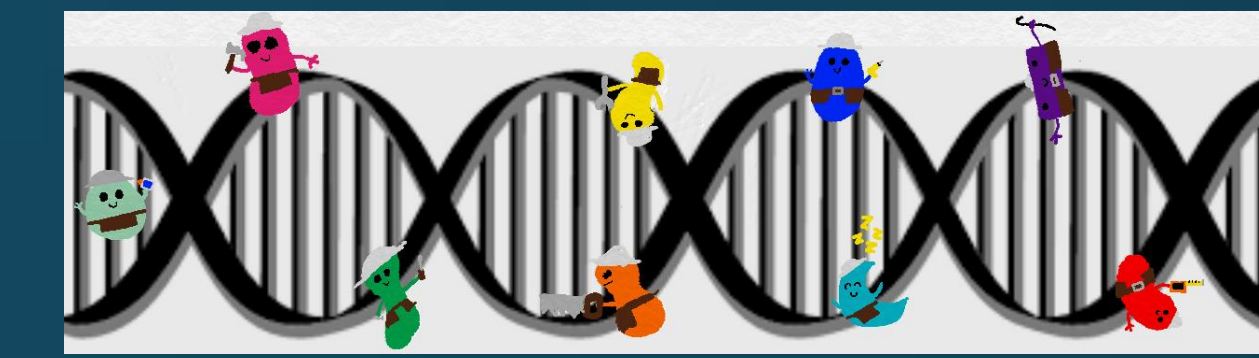


Everyone Needs a Repair Crew: *Elizabethkingia anophelis* R26 DNA Repair



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ABSTRACT

There have been several outbreaks of *Elizabethkingia anophelis* (*E. anophelis*) resulting in 32 deaths (5, 10). Out of nearly 3800 genes of *E. anophelis* we chose the subsection of DNA repair proteins. We found our system of DNA repair through the RAST database and where the DNA repair proteins are in the chromosome through RNA transcription. *E. anophelis* has a DNA repair system that does not appear in operon form, but is spaced throughout the chromosome. Two of our DNA repair proteins showed significant upregulation or downregulation when introduced to the antibiotics.

INTRODUCTION TO ELIZABETHKINGIA ANOPHELIS AND DNA REPAIR

E. anophelis is a bacteria that has led to a disease resulting in numerous illnesses and several deaths across the United States in the past several years. *E. anophelis* is found in the gut of certain mosquitos as well as a human pathogen (5, 10). The danger of the bacteria to humans is its resistance to multiple antibiotics. *E. anophelis* has a circular genome of over 4 million base pairs and over 4 thousand predicted coding sequences. Over 100 proteins have been identified and categorized as resistant to antibiotics.

Five of the proteins on the *E. anophelis* genome ring include DNA repair proteins RadA, RadC, putative helicase, AlkB, and RecN (4). These five proteins are responsible for repairing the DNA of *E. anophelis* when the structure of the DNA has been damaged. The DNA repair proteins were introduced to two different antibiotics and each reacted differently. How these genes reacted to the antibiotics was observed. Also, the proteins are dispersed randomly around the genome. Through research of these five genes discoveries could be made in efforts to better understand the *E. anophelis* bacteria, thus, finding a cure to the disease that is produced from the bacteria.

MATERIALS AND METHODS

For our materials, our group used individual laptops to access the Rast and Blast databases that we used to choose and sequence our genes. We also used google docs so that everyone in the group could see all the information and add to it accordingly. Any other materials used were the various websites, that are cited below, that we used to help us better understand our selected genes and their processes.

For our methods we began by navigating to the Rast database where we chose the pathway DNA Repair. This resulted in five genes associated with DNA Repair from the *Elizabethkingia* R26 Genome. We researched each one of the five genes and wrote interpretations of our findings. Our next task was to determine whether a gene was on or off by using the Blast database. We began this task by first navigating to the RAST database where we went to the list of our five genes and clicked on each one which would take us to a new page where we would then click on "sequence". Once the gene was sequenced we would copy and paste this into the Blast database so we could obtain the FASTA format. We would then select the second option on the drop down bar that translated it into contigs. Finally, we would hit search and copy and paste the results into our electronic notebook. We then deciphered our results. Our next task was to record the transcription pattern we observed in the previous task. In order to do this we navigated to D2L where we were provided and MS excel sheet. We downloaded the table and found our five genes of interest. We then observed and recorded the numbers from the table. We made a new table where we pasted the results from the previous table and deciphered our observations. We elaborated on whether the genes showed no or little to great significant change of transcription. Our second objective within this task was to capture an image of the genes surrounding each of our five genes and to determine whether or not these surrounding proteins are related to our genes of interest. We then deciphered our results.

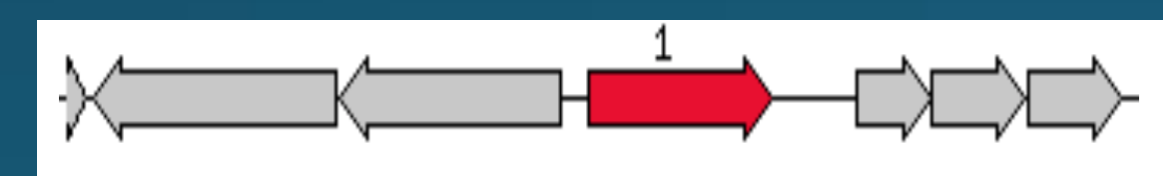
RESULTS

Protein Name	RAST Name
RadA	fig 1246994.5.peg.334
AlkB	fig 1246994.5.peg.468
RadC	fig 1246994.5.peg.853
Putative Helicase	fig 1246994.5.peg.1571
RecN	fig 1246994.5.peg.2381

The RadA protein recombines damaged DNA to DNA helix (3). AlkB protein restores the DNA after damage specifically by free radicals or alkylating agents endogenously generated in metabolism (1, 2). RadC protein repairs damaged DNA from UV and X-ray exposed radiation in prokaryotes or the single celled organisms (8). The putative helicase unpackages an organism's genes for enzyme breakdown (6). RecN protein bridges and holds the strands of DNA apart, holds the strands together, and pulls the DNA strands back together (7, 9).

Protein Name	Fold Change* (Cefotax/Control)	Fold Change* (Imipenem/control)	TOTAL mRNA***	mRNA Counts Control	mRNA Counts Cefotax	mRNA Counts Imipenem
RadA	1.2	1.1	158	48	59	51
AlkB	1.5	-1.7	59	19	29	11
RadC	1.2	-1.2	33	11	13	9
Putative Helicase	-1.4	1.0	287	105	74	108
RecN	1.8	1.1	62	16	29	17

For genes that showed little to no significant change of transcription under the introduced antibiotic (<50% increase/decrease), the antibiotics do not affect the gene. For genes that showed significant change of transcription under the introduced antibiotics (>50% increase/decrease), the antibiotic does affect the gene. The genes that showed a slight change of transcription include: AlkB protein under Cefotax and Imipenem, and RecN protein under Cefotax.



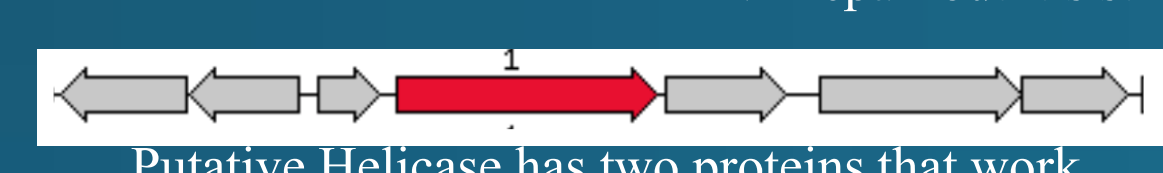
The proteins around RadA perform random functions not making it a part of an operon.



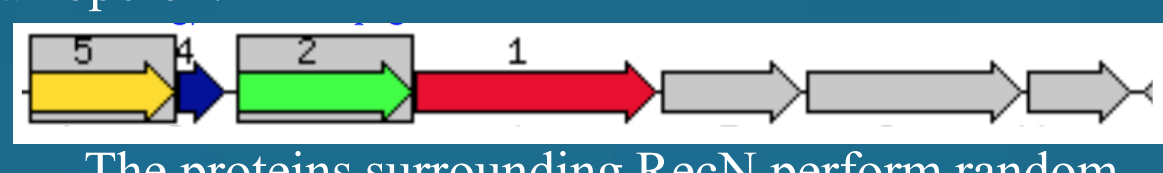
AlkB has one protein related to DNA repair but it is indirectly so making it irrelevant.



RadC has one protein, Polymerase III, that is directly related to DNA repair but it's still unlikely to be part of an operon.



Putative Helicase has two proteins that work together with the rest performing random functions.



The proteins surrounding RecN perform random functions making it not part of an operon.

Each DNA repair protein is surrounded by unrelated proteins. Therefore, it is safe to reasonably conclude that none of the DNA repair proteins belong to an operon. It is also noticed that each of the DNA repair proteins are located at random places on the ring of genes.

DISCUSSION

After examining the five DNA repair proteins it is likely that these proteins contribute to *E. anophelis*' ability to resist antibiotics. The function of these proteins are essential to the survival of the genome itself. Observations on how the proteins react to the introduction of antibiotics as well as how the proteins relate to each other and other proteins on the genome were made. It was found that both the AlkB and RecN proteins upregulated when Cefotax was introduced, and AlkB downregulated under the Imipenem antibiotic. Two of the DNA repair proteins increase in mRNA transcription under these antibiotics, showing these proteins are necessary in *E. anophelis*' resistance to antibiotics.

It is also important to note the location of each of the DNA repair proteins in relation to each other. Each DNA repair protein is randomly located around the genome. This may be due to the importance of the repair proteins. If all the proteins were in one location on the genome the safety of the genome as a whole would be compromised. Dispersing the proteins around the genome helps to ensure the safety of the genome. In addition, proteins surrounding each DNA repair protein were examined to determine if the proteins belonged to an operon. It was found that no operon existed between any of the proteins. More research needs to be done to further understand why the AlkB and RecN proteins reacted as they did to the antibiotics. This understanding can further help explain the *E. anophelis* resistance to antibiotics. Also, researching the makeup of genomes in general can help explain why the DNA repair proteins are randomly dispersed on the *E. anophelis* genome.

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• Visual. DNA strand used for the margin design. Available from: <https://openclipart.org/detail/249955/dna-helix>