Everyone Needs a Repair Crew: Elizabethkingia anophelis R26 DNA Repair

Rachel Mathieson, Casandra Salinas, Morgan Hixon, Audrey Lucas, Patricia Canaan, Nathaniel Torres, William Johnson

ABSTRACT

There have been several outbreaks of Elizabethkingia anophelis (E. anophelis) resulting in 52 deaths (5, 10). Out of nearly 3800 genes of E. anophelis we chose the subsection of DNA repair proteins. We found UV and X-rays are the main factors that cause DNA damage. The 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 bacterium that has lead to a disease resulting in numerous illnesses and several deaths across the United States in past several years. E. anophelis is found in the part of certain mosquitoes 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 genes 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 antibiotic and each reacted differently. Here 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 came from the various websites, that are listed below, that we used to help us better understand our selected genes and their processes.

For our research, we migrated 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 that 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 electrotext 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 iZL where we were provided and MiX 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 significant change of transcription was the result of antibiotic treatment. Our 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

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Fold Change* (Cefotax/Control)</th>
<th>Fold Change* (Imipenem/Control)</th>
<th>TOTAL mRNA***</th>
<th>mRNA Counts Control</th>
<th>mRNA Counts Cefotax</th>
<th>mRNA Counts Imipenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>RadA</td>
<td>1.2</td>
<td>1.1</td>
<td>158</td>
<td>48</td>
<td>59</td>
<td>51</td>
</tr>
<tr>
<td>AlkB</td>
<td>1.5</td>
<td>-1.7</td>
<td>59</td>
<td>19</td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td>RadC</td>
<td>1.2</td>
<td>-1.2</td>
<td>33</td>
<td>11</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Putative Helicase</td>
<td>-1.4</td>
<td>1.0</td>
<td>287</td>
<td>104</td>
<td>74</td>
<td>108</td>
</tr>
<tr>
<td>RecN</td>
<td>1.8</td>
<td>1.1</td>
<td>62</td>
<td>16</td>
<td>29</td>
<td>17</td>
</tr>
</tbody>
</table>

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.

For our discussion, we discuss the results of the DNA repair proteins and how they can affect certain bacteria. The DNA repair proteins are used to fix DNA damage so that bacteria can grow and multiply. Our group has shown many different proteins that are associated with DNA repair. This is very important to the field of medicine because of the importance of these bacteria.

REFERENCES

2. RAST [Internet]. Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, 02454
7. Protein Data Bank [Internet]. Protein Data Bank; 2016 [cited 2016Nov15]. Available from: http://www.rcsb.org/pdb/