Katherine Filpo Lopez
December 17th 2020
Professor Kerri-Ann
Final Project Report: Bacteria and Antibiotics

Introduction

In the human body, there are billions of bacteria. These bacteria can help the body do things like digest food or help your immune system. However, when the bacteria are affected in some way, it can have adverse effects on the body. These issues are treated with antibiotics. However, some things cannot be treated effectively with these antibiotics. Stomach issues like *Escherichia coli*, or *E. coli* infections can be painful and difficult for people to go through, and because E. coli is an ESKAPE pathogen the antibiotics we have cannot treat this problem easily. These microbes can infect up to 2.8 million people just in the United States, and can kill more than 35,000 people (CNN 2020).

ESKAPE pathogens are bacteria that are highly virulent and antibiotic resistant, so treatment for these infections can be impossible. The bacteria in the ESKAPE group are as follows: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp*. *E. coli* falls under the *Enterobacter spp* category, and the lack of treatment means that scientists have to turn to other things to help.

One such thing that scientists have turned to is, surprisingly enough, soil. There are billions of bacteria in soil that are undiscovered that may function as antibiotics by inhibiting the growth of ESKAPE pathogens. In my Microbiology class, my group and I decided to collect bacteria from different sites and study them for their use as ESKAPE pathogen inhibitors. For this project, the samples were collected on Bard College's campus and the different sites were picked based on whether or not they had a type of fungus within one foot of the site. We tested our library of about 50 bacteria from our sites against the ESKAPE pathogens or ESKAPE

pathogen variants that were safe to be around. Then once we narrowed down our library to about 7 effective bacteria (effective meaning that the bacteria inhibited the growth of at least 2 ESKAPE pathogens), we sent these bacteria off to a lab for sequencing.

Methods

The code I used was written by Professor Kerri-Ann. I used the Neighbor-Joining Tree code and the MultipleSequenceAlignment. I also used a 4Peaks package to open the data that I received from the Cornell sequencing, and BLAST them to find the bacteria that they were most like.

I used the MultipleSequenceAlignment code, specifically the pairAlignScore() method to calculate the distance matrix for each bacteria to each other bacteria. After I got the distance matrix, I put the matrix into the Neighbor-joining code and found the tree.

I have two trees because the sequencing I used had some differences. The Cornell lab ran the samples they received from us, about 30 or so from the class and most went really well and had very clear results. However, there were about 6 samples, from my group and the other class group that did not come out well, so they re-ran them and sent those to us as well. For my group, the samples that were re-run were A4 and A5. Due to the slight differences in sequences, I ran two Neighbor-Joining trees with slightly different matrices.

Figure 1. A clear sequencing of A1

Figure 2. The first run of the sequencing of A4

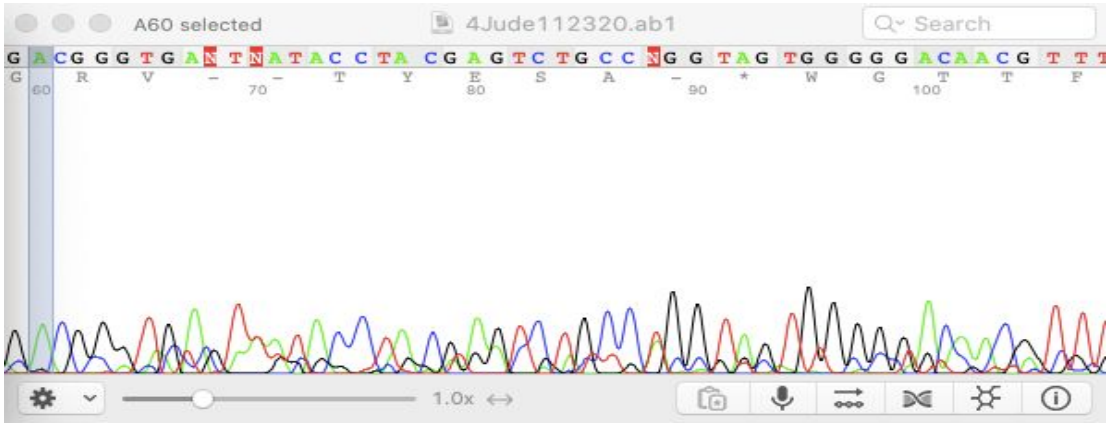

Figure 3. The second run of the sequencing of A4. In this figure, the sequencing is dramatically different in this section.

I thought about using the BLAST sequencing results to add the species that the bacteria were most like but I got a 96% accuracy for most of the bacteria (the one exception was bacteria A5, which was 85% accurate), and due to this, I knew that if I downloaded the bacteria that they were most like, I would just get pairs of the sequence that the bacteria was most like and the bacteria in question, and this is not what I wanted to see, as this is already information I know.

I also used the multiple alignment code to see how all the sequences would align, but got a result that I deemed unnecessary for analyzing the sequences.

Results

The trees were not as similar as I expected them to be when I ran them with the Neighbor-Joining code. Even though I knew that A4 and A5 were different sequences, when I used NCBI to BLAST them, I got the same results for both sequences.

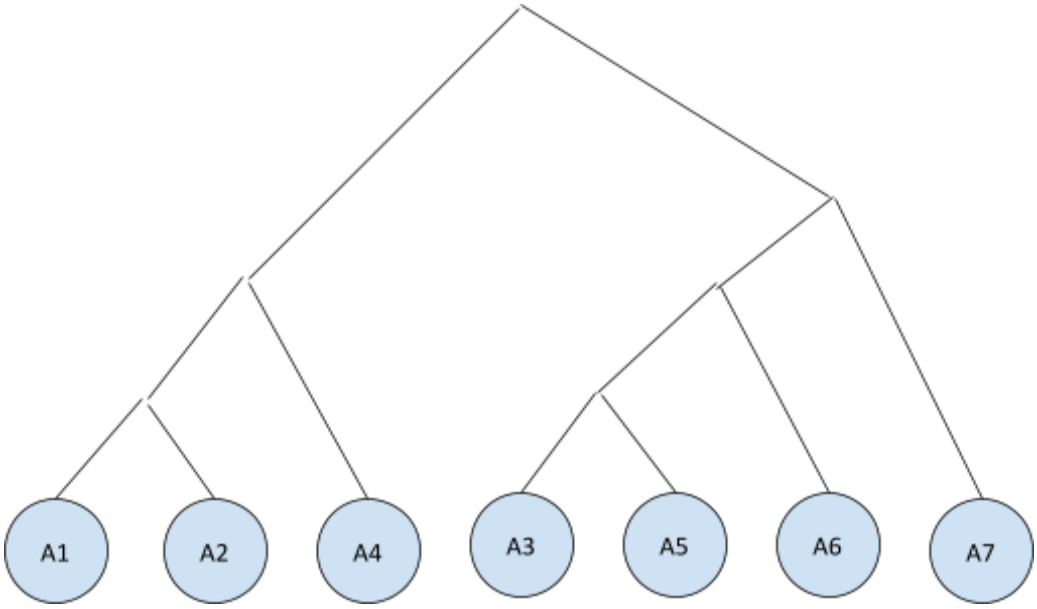

Figure 4. The tree with the first version of A4 and A5.

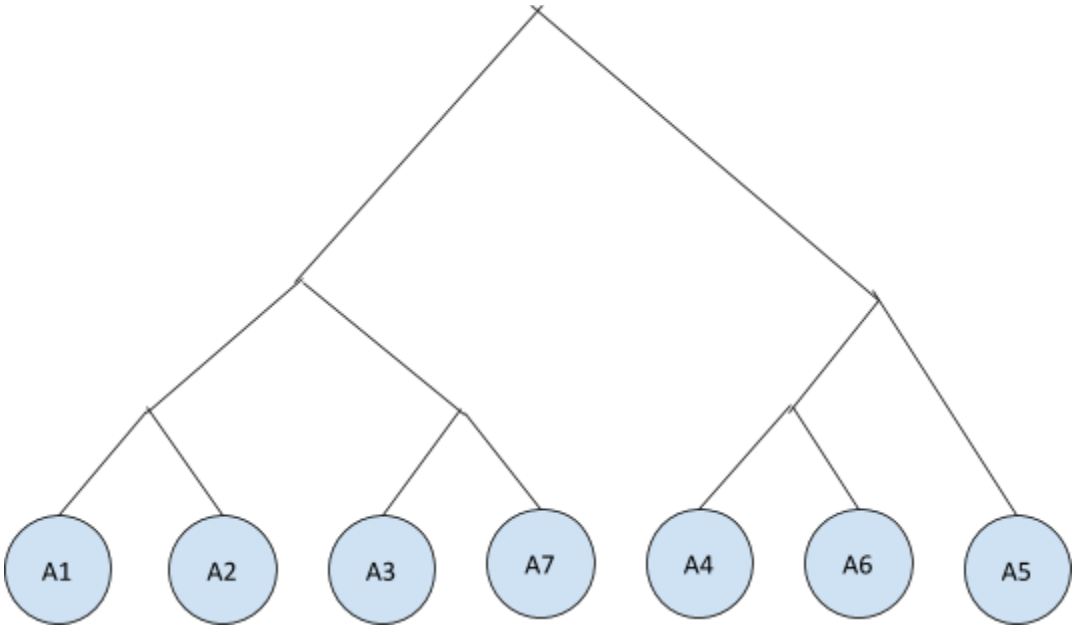

Figure 5. The tree with the second version of A4 and A5

The reason I thought the trees would be similar when the BLAST results were similar is because if they were drastically different sequences, then the BLAST would say that the bacteria did not match with the same bacteria because the sequences would be so different. But the reason that the trees must be so different even though the BLAST results are the same is because this is a closed system that only has 7 sequences, and without more data to have, the different sequences (which are all different bacteria from each other) must have a drastically different nucleotide content, from one version of A4 and A5 to the other, instead of slight differences that I thought there would be instead.

Nevertheless, I find it interesting that A1 and A2 stayed the same, while all the other sequences moved and paired differently.

Conclusion

The lab went well.

Credit

CNN JG. Turning to dirt for antibiotics in the fight against superbugs. CNN. [accessed 2020 Dec 17]. https://www.cnn.com/2020/10/06/world/dirt-new-antibiotics-wellness-scn/index.html.

Professor Kerri-Ann Norton for code