Analysis of 16SrRNA Gene Mutations on Antibiotic Resistance in E Coli

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Abstract

Understanding more about the mechanisms behind anitbiotic resistant genes will help us identify new solutions to this pressing problem, as well as uncover more areas of the microbial world that can be better understood within the scientific community and contribute to future findings. In this experiment, our goal was to learn more about the development of antibiotic resistance and evaluate the gene sequence of a cell that has undergone a mutation. We predicted and found a correlation between mutations in the 16SrRNA sequence and resistance to Tetracycline in E Coli.

Introduction

Antibiotic resistance in bacterial cells is the ability to resist the effects of a given treatment designed to kill bacteria [1]. The significance of antibiotic resistance is the inability to effectively treat pathogenic bacteria with the same type of antibiotic, and the possibility of a bacteria becoming resistant to all known forms of treatment [1].

Tetracycline is a group of antibiotics that target the ribosome of bacterial cells. The exact target of tetracycline to the 30S subunit is within the bacteria's 16SrRNA gene, preventing the translation of proteins [2]. By performing PCR of this gene, one would be able to potentially see variants in nucleotide sequences from a bacterial cell that has mutated to become resistant to this medication. Additionally, a lack of sequence variability between a mutated and non-mutated bacteria could potentially indicate that the storage of this mutation within the cell's plasmids, rather than within the nucleoid region.

We investigated if mutations in the 16SrRNA gene are correlated to tetracycline resistance in E Coli. Our hypothesis was that the more resistant the bacteria would be to the antibiotic, the more mutations in the gene. To test this, we created progressively more resistant bacteria and analyzed the 16SrRNA sequence for them through PCR.

Methods

Materials

NA, Tet1.5, Tet3, and Tet6 plates, micropipettes, centrifuge, vortex, hot water bath, agarose gel, thermocycler, gel box, hot shot, GoTaq Polymerase, TAE buffer, Master Mix with 320F and 817R primers, E. coli, Bunsen burner, loop, and biosafety cabinet

Location

Lab: *Whatcom Community College*, Kulshan Hall Biology lab room 108, 1/8/25 - 3/28/25

Procedure

With our provided gram negative E-Coli, we plated the bacteria on TetNA, Tet1.5, Tet3, and Tet6 agar plates consecutively. We took samples from the previous level of antibiotic resistance to grow increasingly more resistant bacteria. Samples were taken from each plate and lysed to replicate the 16srRNA gene. We performed gel electrophoresis of each sample to see if our samples could be sent off for sequencing. We sent the bacterial cells off to a sequencing company to perform bioinformatics.

Sequences

NA	Tet 3
NNNNNNNNNNNNGANNNNGCACAATGGGCGCAAGCCT GATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTT GTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAA TACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGG CTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCA AGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGC GGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTG GGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAG GGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAG ATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGAC GAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAA CAGGATTAGATACCCTGGTAGTNNNNCNGNNAAAANGA	GNNNNNNNTNNGGGNNNNNGNNNNTGGGNNNAGNN TGATNNANNNNNGNNGNGNGNATGAAGAAGGNCTNNN GGNTGTAAAGTACTTTNAGCGGGGAGGAAGGGAGTAAA GTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCA CCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGG TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGC AGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAA CCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTA GAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTA GAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG GACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGC AAACAGGATTAGATACCCTGGTAGTNNN

Table 1. This table includes the two sequences we got from sending our PCR samples to a sequencing company.

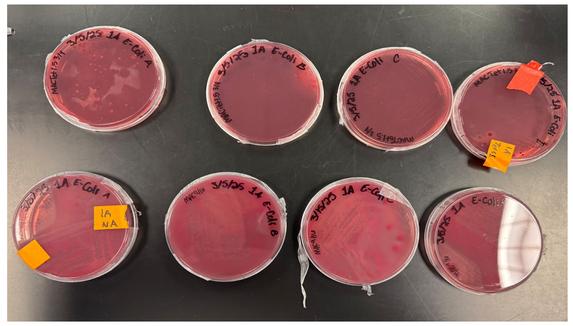

Figure 1. The growth of E Coli in the presence of different quantities of tetracycline.

Phylogenetic Tree of Tet3 and NA E-Coli

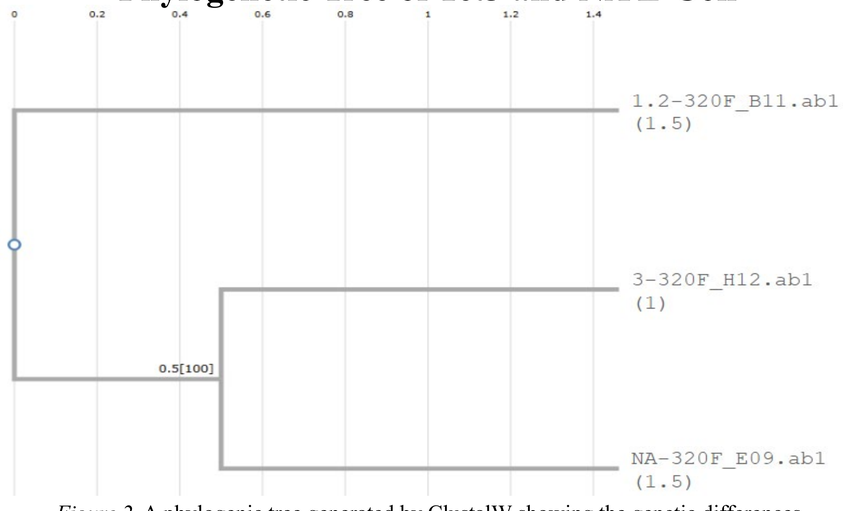

Figure 2. A phylogenic tree generated by ClustalW showing the genetic differences between the Tet3, non-resistant E Coli, and Salmonella.

Discussion

As you can see from the visual growth of colonies on our plates, we were able to create bacteria resistant to 1.5 times (Tet1.5) and 3 times (Tet3) the level of tetracycline they should be able to survive. Through the results of sequencing, we found that there are some differences in the 16SrRNA gene between the non-resistant and Tet3 resistant bacteria. By doing a BLAST search of both sequences we found a 2% difference in the percent identity of the 16S gene in the database between the non-resistant and Tet3 resistant bacteria. By comparing it to a salmonella sequence we can see that they are still closely related but the sequence did mutate as it became more resistant to antibiotics.

Our data shows a potential correlation between mutations in the 16SrRNA gene and tetracycline resistance [3]. Since we know that tetracycline targets the 30S subunit with the 16SrRNA gene, we can conclude that mutations in this gene may be what is creating this resistance [2]. We are unable to say whether the gene gets more mutations as the level of resistance increases because we were only successfully able to do PCR for the non-resistant, and Tet3 resistant, but not the Tet1.5 resistant bacteria. Our PCR was unsuccessful because we were unable to extract the DNA needed to send off for sequencing.

Our findings have started to answer our research question, but further research would need to be done to see if the number of mutations increases as the amount of resistance increases. This could be done by continuing to create levels of resistance and analyzing sequences from the created samples.

Acknowledgements

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References/ Work Cited

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