Transferring Tetracycline Resistance Between an Unknown Bacteria and *E. coli*

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Abstract

We are experiencing a global health crisis due to a rise of antibiotic resistant infections. Through both overuse and misuse of antibiotics, resistance continues to spread making drug treatment ineffective and resulting in the death of at least 700,000 people worldwide each year (Mancuso et al., 2021). We predict the unknown antibiotic resistant bacteria will have an ecological relationship with domestic horses because the soil sample tested for tetracycline resistance was taken from a horse stall. Additionally, we predict we will be able to successfully perform horizontal gene transfer of the antibiotic gene from the unknown bacteria to *E. coli* as evident by growth on a MacConkey Agar plate. In summary, we successfully identified the unknown but were unsuccessful in transferring the antibiotic gene, thus refuting our first and inconclusively answering our second hypothesis.

Introduction

Antibiotic resistance continues to rise at alarming rates through both vertical gene transfer of daughter cells and horizontal gene transfer from one cell to another, posing a significant risk to human and agricultural health. This is because the use of antibiotics "kills sensitive bacteria but selects for growth of those few organisms that may be resistant" (Genné-Bacon et al., 2018). Therefore, by researching this subject the medical community can begin to better understand and defend against the spread of antibiotic resistance. In order to further research this topic, we asked the following two research questions: can we identify unknown antibiotic resistant bacteria from colonies grown on a 30µL/L tetracycline agar plate (Tet30) and can we transfer antibiotic resistant from unknown tetracycline resistant bacteria to competent *E. coli*?

We predict the identified unknown bacteria will have an ecological relationship with domestic horses because the soil sample tested for tetracycline-resistance was taken from a horse stall. Additionally, according to Kroemer (n.d.), "*E. coli* made competent either through CaCl2 and heat-shock or through electroporation will have better membrane permeability (pores), enabling plasmid uptake." Therefore, we predict we will be able to successfully perform horizontal gene transfer of the antibiotic gene from the unknown bacteria to *E. coli* as evident by growth on a MacConkey Agar plate because we will be using store bought competent *E. coli.*

Methods

Materials:

NA and Tet30 plates, micropipettes, centrifuge, vortex, hot water bath, agarose gel, thermocycler, gel box, LB Media nutrient broth, GoTaq Polymerase, TAE buffer, Master Mix, shaking incubator, *E. coli*, bunsen burner, loop, and biosafety cabinet

Location:

Lab: Whatcom Community College Kulshan Hall biology lab room 108, 2/19/24 - 3/13/24

Soil sample location: 48°00'46"N 121°54'05"W horse stall, 2/4/24

Procedure:

Perform antibiotic resistance soil sampling according to the prevalence of antibiotic resistance in the environment (PARE) project (Genné-Bacon et al., 2018). Sample colonies from Tet30 agar plates. Lyse sample cells and perform PCR to replicate the 16S rRNA gene. Perform gel electrophoresis of each sample's replicated DNA to identity bacterial cells. Send bacterial cells off to a sequencing company to perform bioinformatics. Lyse bacteria cells to isolate plasmid DNA. Expose competent *E. coli* to bacterial plasmids. Perform gel electrophoresis of control group *E. coli* and exposed *E. coli*. Plate treated *E. coli* on a Tet30 agar plate. Observe and record results.

Tetracycline Resistant Bacteria Species

Name	*S. rhizophila*
Identities	436/437(99%)
Gaps	1/437(0%)
E-value	0.0

Table 1. This table provides a summary of our results gathered from blasting our sequences. The important values are shown above.

Gel Electrophoresis Results

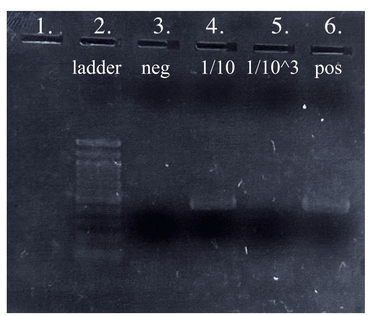

Figure 1. This figure shows the results to a gel electrophoresis run of replicated 16S rRNA genes from unknown tetracycline resistant cells. Bands are visible in well 4 in addition to the positive control and DNA ladder.

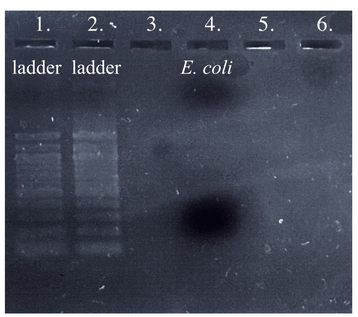

Figure 2. This figure shows the results to a gel electrophoresis run of *E. coli* exposed to tetracycline resistant plasmids from unknown bacteria. No bands visible aside from the DNA ladders.

Discussion

Two colonies from different Tet30 agar plates were sampled, one from a 1/10 dilution and one from a 1/1000 dilution, but only the first was replicated by PCR of the 16S rRNA gene, as shown in Figure 1. The resistant bacteria was identified as *Stenotrophomonas rhizophila* strain H5a96, as shown in Table 1. This result refutes our first hypothesis as *S. rhizophila* is not associated with domestic horses but is rather a symbiotic plant bacteria. Unfortunately, horizontal gene transfer of antibiotic resistant plasmids to competent *E. coli* was unsuccessful. Gel electrophoresis of exposed *E. coli* showed no bands of plasmid DNA, as shown in Figure 2, and no growth was present when later transferred onto Tet30 agar plates. Therefore, these results fail to support or deny our second hypothesis.

Gel electrophoresis of unknown colonies in Figure 1 show only one lane, indicating the other sample was not a bacteria containing the gene targeted for replication. Moreover, Mancuso et al. (2021) states other factors, including "poor community hygiene, safer food, poor infection control in hospitals and clinics, accumulation of antibiotics in the environment and their use in the animal and food industries" are responsible for the increase in antibiotic resistance prevalence. Therefore, the identified bacteria could have become antibiotic resistant from the horses so some relationship may still be evident.

Time and funding were significant limitations to our research and could be improved in the future. When complications arose including sampling a colony which was not a bacteria or unsuccessful plasmid transfer, funds prevented us from sampling a new colony and time prevented us from attempting plasmid transfer again. Peers were successful in plasmid transfer indicating the process was feasible and additional time might have allowed us to gather more meaningful results.

Acknowledgements

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References

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