Tetracycline-Resistance in Bacteria Colonies From Soil From Different Socio-Economic Locations

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Abstract

My purpose was to test if burning soil from different areas of the same city would yield similar results in the amount of tetracycline-resistant (Tet^R) bacterial colonies reduced in the burnt samples. The data presented in my findings supports both of my hypotheses, positively pertaining to the sterilizing abilities of an open flame and the inverse correlation between Tet^R bacterial concentration in soils and socio-economic conditions in which the soil was gathered. This study showcases how we can better utilize flame sterilization for the soil in community gardens, which are utilized by the local public as a food source.

Introduction

In the field of microbial cultivation, fire is a well-utilized sterilizer of tools and surfaces. For example, inoculation loops are flamed before and after collecting samples from bacterial colonies, to utilize aseptic technique and not skew the data in laboratory experiments. (Anonymous, 2020) In addition to sterilizing tools inside the laboratory, fire can sterilize biological matter, such as the soil from our local communities (Xiang, 2014). In this experiment, I tested the sterilizing properties of fire on soil from different socio-economic regions of the city of Bellingham, to discover if burning the soil would have a significant impact on reducing harmful bacteria that could contaminate residents of the city. My hypothesis was that burning the soil will reduce the biodiversity of microorganisms in the soil, therefore significantly reducing the population density of bacteria in the soil. My additional hypothesis was that soil from a lower-income environment would contain higher quantities of antibiotic-resistant bacteria than soil from a higher-income area of the same city.

Methods

Materials & Location

Materials:

2 Soil Collection Tubes and trowel sterilized with 70% isopropyl alcohol
Micro spatula sterilized with 95% ethanol
20 test tubes with 9ml sterilized water
Test tube rack
Vortex
44 petri dishes with MacConkey agar base
Parafilm to seal petri dishes
Butane torch and 2 crucibles to burn the soil samples

Locations:

Whatcom Middle School, 48.7590° N, 122.4803° W
Bellingham Food Bank, 48.7546° N, 122.4719° W
Kulshan 108 Biology Lab Room

Procedure

Starting this experiment on 3/1/2024, I collected soil samples with a trowel from two local sources; Whatcom Middle School (suburban and middle-class environment), and the Bellingham Food Bank (low-income environment). After I brought my samples to the lab, I replicated the PARE procedure (Genné-Bacon et. al. 2018), plating my soil samples in 22 MacConkey agar petri dishes; 11 for the Bellingham Food Bank soil, and 11 for the Whatcom Middle School soil. I left the petri dishes to sit out in the lab room temperature (22°C). 48 hours later, I burnt the leftover soil samples with a butane torch in two separate crucibles for a duration of 10 seconds for each flame. I repeated the PARE procedure with 22 more petri dishes, using 1g of each of the burnt samples. 125 hours later, I counted the visible bacterial colonies by dotting them with a pen and recording the numbers on a clicker.

Percent Tetracycline-Resistant Cells

	Unburnt BFB Soil	Unburnt WMS Soil	Burnt BFB Soil	Burnt WMS Soil
Control Plate CFUs	3.05 CFU/gram soil	5.1 CFU/gram soil	0	0
3 µg/ml Tet CFUs	86 CFU/gram soil	27.5 CFU/gram soil	0	0
30 µg/ml Tet CFUs	0	0	0	0
Volume Plated (ml)	0.2	0.2	0.2	0.2
% TetR CFUs	28.2	5.4	0	0

Table 1. A table summarizing the counts of Colony-Forming Units (CFUs) per each gram of soil that has been diluted and plated in the experiment.

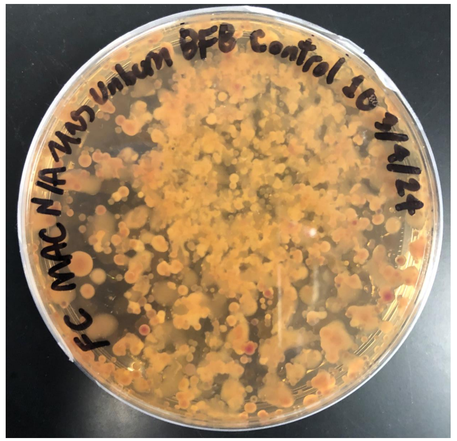

Figure 2. MacConkey Agar petri dish containing bacterial colonies cultivated from the Bellingham Food Bank soil. No traces of tetracycline are present on this plate, so one cannot accurately determine what colonies are tetracycline-resistant unless tetracycline is added.

Figure 2. (Left) Lab Technician Bethany Tegt burning the Whatcom Middle School soil sample with a butane torch. (Right) Burnt soil sample contained in crucible.

Discussion

Through my research, I found significant Tet^R bacterial growth on the petri dishes containing dilutions from the soil samples that I left unburnt. From the countable colony-forming units (CFUs) I have recorded for both dilutions, 28.2% of the CFUs from the unburnt Bellingham Food Bank dilutions contained Tet^R bacterial growth, compared to only 5.39% of the unburnt Whatcom Middle School dilutions containing Tet^R bacterial growth. None of the petri dishes containing the burnt soil dilutions presented colony-forming units, thus no bacteria was present, confirming my main hypothesis. My additional hypothesis was also supported by the fact that the Bellingham Food Bank soil contained over 5 times the percentage of Tet^R bacterial growth than the Whatcom Middle School soil, confirming the disparity between low-income and middle-class environments.

From my data, burning the soil kills the bacteria indiscriminately in a manner that tetracyclines cannot replicate. Tetracyclines are renowned for being broad-spectrum agents, culling a variety of bacteria regardless of Gram stain criteria or other characteristics. Unfortunately, they run into the problem of bacteria that have developed immunity to the antibiotic. (Grossman, 2016) On the other hand, burning the soil killed all the bacterial CFUs, regardless of tetracycline resistance. Burning of bacterial cells denatures their proteins, rendering their antibiotic resistance irrelevant as the genes encoding the resistance lose their function under these high temperatures.

On a socio-economic standpoint, citizens living below the poverty-line experience higher risk of infection from antibiotic-resistant bacteria. (Gilbert, 2006) The burning of soil in community gardens, prior to nutrient replacement and planting, will further ensure that citizens below the poverty line will have access to hygienic sources of food. Additional research I would suggest is on whether having burnt soil as a blank slate for the addition of nutrients would yield crops as healthy as those grown in unburnt soil with its original remaining bacteria. Extenuating this research on a city-wide level would aid the community efforts to maintain the sustainability and access to food for lower-income citizens.

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Acknowledgements

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