THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

The Effect of Adding Bacteria-Rich Samples on Colony Growth in Soil

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Abstract

The quantity and diversity of bacteria within soil plays a tremendous role in the environment. A way to observe the overall effects of these bacterial species on a soil sample can be distinguished through biofilm formation. Biofilm is a substance that bacterial cells secrete, which serves different purposes, protecting colonies from their environment and anchoring colonies to surfaces. Bacteria secrete biofilm in response to environmental stimulation, including exposure to different types of bacteria (1). The purpose of the course BIOL 102L was to aid Dr. Shank at the University of Massachusetts in her biofilm research by screening different soil samples to search for microbes that might cause *Bacillus subtilis* to produce biofilm (1). Bacteria were obtained from soil samples from different places across UNC's campus. The soil was then treated with substances that we believed would promote the diversity of bacteria found in each treated soil sample, such as compost juice, probiotic pills, eggshells, and beer. The treated and untreated samples were diluted and plated onto *B. subtilis* reporter, which would fluorescence if our soil bacteria produced biofilm. None of our samples showed true fluorescence, but some of our soil treatments displayed an increased diversity of bacteria in the treated samples.

Introduction

Microbes are essential to many biological processes in the environment, such as promoting growth and resistance within plants, and helping promote nitrogen fixing. Biofilms produced by microbe colonies are an important topic of study in biology, as they play a role in many bacterial infections (5). *B. subtilis* is one of such disease-causing bacteria which produce biofilm. Through this research project, we hoped to be able to promote bacterial growth within soil samples in order to uncover new bacteria types that cause a *B. subtilis* sample to produce biofilm.

- We hypothesized that all of our treatment methods, which involved the introduction of additional bacteria into the soil that we collected would induce bacterial growth in our samples.
- We then concluded that as bacterial growth increases, there would be a higher probability that there would be bacteria that act as biofilm inducers upon *B. subtilis.*

This allowed for the identification of potential biofilm inducers through the observation of fluorescence within the bacterial lawn.

References and Acknowledgments

Thanks to Dr. Barbara Stegenga and Dr. Christina Graves for guiding this research project in BIOL 102L. This work is supported by the UNC Biology Department.

(1) Barbara Stegenga, (2022). BIOL 102L Lab Manual

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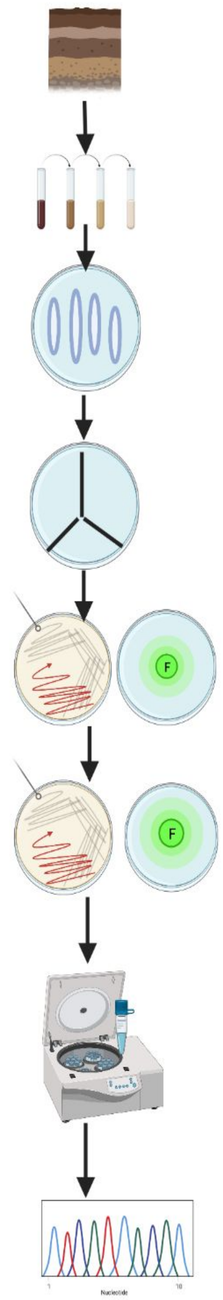
Materials and Methods



1. Soil was harvested from different locations on UNC's campus
2. Half of the collected soil was treated, the other half was left as an untreated control. (treatments: probiotic, compost juice, eggshells, and beer)
3. Treated and untreated samples were diluted with the serial dilution method.
4. Drop-tilt method: dilutions were plated and colony forming units (CFU's) were counted.
5. Co-culture plates were made with three sections: a control section with only *B. subtilis* reporter, *B. subtilis* reporter with the treated sample, and the reporter with the untreated sample.
6. Co-cultures were examined for fluorescence (biofilm)
7. **Secondary Screening** to verify our original findings and confirm the presence or absence of inducers
8. Genomic DNA prep- we isolated the DNA from one of each of our bacteria samples
9. **Amplified 16S sequence**, a conserved, genetic commonality between all bacteria, using PCR Identified bacterial identity based on the sequencing using BLAST and MEGA

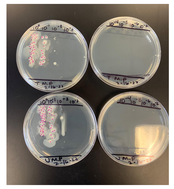


Figure 3: The drop-tilt method implemented by Michelle Pantaleon

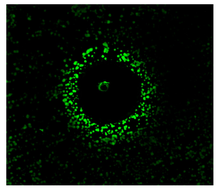


Figure 4: A sample of *B. subtilis* that fluoresces when it produces biofilm. Source: (1).

Results

Treatment Types	Treated Samples Bacteria Count (CFU/mL)	Untreated Samples Bacteria Count (CFU/mL)	Presence of Fluorescence
Probiotic	2.8×10^3	2.0×10^3	None
Compost Juice	7.5×10^6	3.73×10^6	None
Eggshells	70	90	None
Beer	69	73	None

Figure 1: This table delineates the calculated amount of colony forming units of bacteria for each treatment type and whether fluorescence was detected after completing the drop-tilt method.

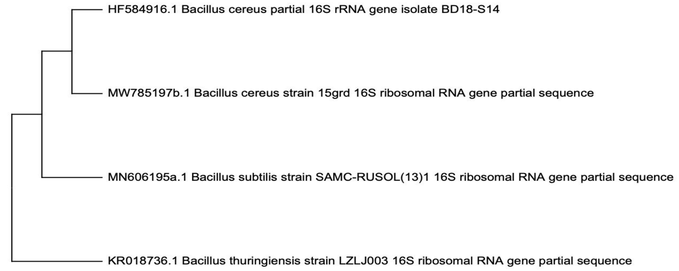


Figure 2: The diagram above represents a phylogenetic tree of the four main bacterial strains found in our soil samples and their relation to each other.

After counting CFUs, it was apparent that the beer and egg shell treatments did not increase bacterial growth, while adding compost juice and probiotic to the soil did increase growth. Figure 2 shows that each of our picked bacterial strains were of the *Bacillus* genus, which are commonly found in soil.

Discussion

Our hypothesis was not supported for all treatment types. The reduced bacterial growth seen in the eggshell and beer-treated soils could be attributed to the fact they are not as rich in bacteria as probiotics and compost juice are. For beer, it is possible that the alcohol in the sample killed some of the bacteria in the soil. We hypothesized the calcium within the eggshells would lead to increased bacterial growth, however, our results do not support this. We believe that the decrease in bacterial growth could be due to a confounding variable, such as some other species present in the soil and the excess yolk left from the eggshell. To further isolate this variable in the future, we could add calcium in the form of a dissolved pill to a soil sample and observe its effects on bacterial growth. The probiotic treatment was expected to increase the diversity of bacteria in the soil sample, as probiotics include microorganisms that promote human health (2). The effect of probiotics on soil bacterial growth should be further studied.

Our second hypothesis was not supported either because none of the secondary screenings indicated that we collected a true biofilm producer, including for the samples that showed increased bacterial growth. In the future, we will try different treatment methods, such as compost juice with more bacteria-rich components, concentrated yeast and calcium samples, and different types of probiotic pills.