Treatment of Soil Bacteria with Sweeteners

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Abstract

In this project, we tested the effects on the growth of soil bacteria after treatment with commercially available sweeteners, including artificial sweeteners, juice drinks, and energy drinks. It was hypothesized that these treatments would increase growth as the treatments provide nutrients for bacteria growth, excluding artificial sweeteners, which we predicted would decrease growth. Bacteria was obtained from the soil around UNC Chapel Hill campus. These samples were homogenized and then treated with different sweeteners. First growth was evaluated using a serial dilution. It was found that treatment significantly increased CFU/mL for every treatment except artificial sweeteners. Furthermore, the ability of the soil bacteria to form biofilm was tested by examining for the presence of fluorescence in a coculture plate with *Bacillus subtilis*. Coculture plates were created with the different soil samples before and after treatment, and possible inducers were identified. These bacteria then underwent a secondary screen where they were grown in isolation with *B. subtilis.* It was found that only one sample, treated with juice mix, can induce fluorescence and, therefore, induce biofilm formation. DNA sequencing was also performed to create a phylogenetic tree of samples.

Introduction

As a group, we collectively wanted to examine how sweeteners specifically would impact bacterial growth. In our treatments, we assessed the impact a Celsius energy drink, a Caprisun, and Sweet n' Low sweetener will have. The reason we were interested in this is because we believed the nutrients present in the sweeteners used would provide bacterial growth.

Hypothesis

By treating bacteria with sugar solutions, the growth should increase.

Methods & Materials

1. After gathering soil and substances that it would be treated with (Capri Sun, Celsius Energy Drink, Sweet n' Low), 10 frozen aliquots were made for each sample (treated & untreated)
2. Serial Dilutions & Plating/Counting CFUs: made bacterial concentrations lower and easier to count, counted bacterial colonies
3. Co-culture plates, screenings, & biofilm inducers: made plates that isolated untreated and treated samples with reporter (cell of *B. subtilis* biofilm) to identify fluorescence (visibly-lit bacteria that caused biofilm growth in surrounding areas), secondary screening was to confirm samples that experienced bacterial growth
4. Genomic DNA prep, sequencing, & phylogenetic tree: made sequences from DNA to compare relatedness of sample sequences

Results

It was found that all treatments increased CFU/mL except artificial sweetener (See Table 1)
Only treatment with Capri Sun resulted in biofilm formation through the presence of fluorescence
A phylogenetic tree was created using blast of the samples(See Figure 1)

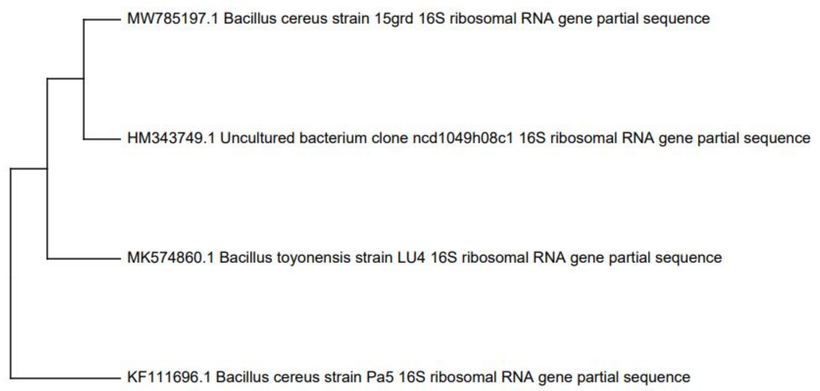

Figure 1. Phylogenetic tree of samples

Discussion and Conclusion

In conclusion, we were able to test our hypothesis to anticipate bacterial growth from treating bacteria with sugar solutions, and found data that supported and opposed it. We found that soil treated with the various sweeteners, with the exception of the artificial sweetener Sweet n' Low, saw an increase in bacterial growth. Only one of the three treatments also produced fluorescence through biofilm formation. This may call for further research on why certain artificial sweeteners might not cause an increase in bacterial growth that are treated in soil, and if it is a nutrient for bacteria. Further research can also be conducted on artificial sweeteners' relationship to *B. subtilis,* as all of our samples were variants of it. This data may be helpful for understanding how pollution of these beverages in our soils could affect our ecosystems as well, depending on the soil environment.

Table 1. Table of treatments, CFU counts, and biofilm formation

Treatment	Untreated CFU/mL	Treated CFU CFU/mL	Biofilm Formation
Celsius Energy Drink	4900	67000	No
Caprisun	400	1000	Yes
Sweet n' Low	40667	17000	No

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