The Effect of Caffeine on Soil Bacteria Proliferation
Madison Grigsby, Mackenzie Inman, Siobhan Morris, and Elizabeth Teka
Biology 102L, Biology Department at the University of North Carolina at Chapel Hill

ABSTRACT: The goal of our research was to examine the effect of caffeine on bacterial growth in soil. We hypothesized that adding caffeine to a slurry of soil bacteria would cause less bacteria to grow than it would without it, due to caffeine’s antibiotic effect against certain strains of bacteria. To test this, we collected four different samples of soil from around campus and made a slurry to put the soil bacteria in solution. We plated both the treated and untreated samples to compare the growth. We additionally added a lawn of Bacillus subtilis reporter to both plates and observed it under a blacklight to determine if any of our bacteria were causing biofilm formation in B. subtilis, characterized by fluorescence around a soil microbe. To confirm if this soil microbe is a biofilm inducer, we picked it and plated it on a secondary screen of B. subtilis lawn. Additionally, we sent our possible inducers to a sequencing facility and blasted it on a database to see which soil bacteria we picked. Finally, we sequenced our bacteria and created a phylogenetic tree to observe that all four of our strains were closely related by a common ancestor. From our experiment we do not have enough information to accept our hypothesis.

BACKGROUND:
Caffeine is a stimulant found in many compounds including coffee, tea, sodas, cocoa, and much more. Its effects include increasing heart rate by stimulating the central nervous system, which can make its user feel alert and more energized. In past experiments it has been shown that caffeine has an antibiotic effect on Escherichia coli. This is important to modern medicine because bacteria are gaining resistance to antibiotics. We wanted to further test this hypothesis with other strains of bacteria that we gathered from the soil. Secondly, bacterial communications is an emerging field of microbiology that studies how bacteria work as a unit to both produce and respond to chemical signals. One of the ways bacteria can respond to chemical signals is by producing biofilm, a protective barrier that can be resistant to antibiotics. Disrupting these signals causes the bacteria to lose the ability to secrete toxic compounds that cause infections. Thus, understanding how to disrupt bacterial communications is the key to developing new medicines and technologies to effectively target bacteria as groups instead of as individuals.

HYPOTHESIS:
- If we add coffee, tea leaves, Coca-Cola, and a caffeine pill to soil samples, the amount of bacteria growing will be less than the samples without caffeine.
- Caffeine is known to have an antibiotic effect on E. coli.

RESULTS:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peach Tea Leaves</td>
<td>Decreased growth</td>
</tr>
<tr>
<td>Brewed Coffee</td>
<td>Decreased growth</td>
</tr>
<tr>
<td>Coca-Cola</td>
<td>Increased growth</td>
</tr>
<tr>
<td>Caffeine Tablet</td>
<td>Increased growth</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSION:
- Through the four treatment methods that each individual selected, there was found to be confounding results. The treatment of Coca-Cola and a caffeine tablet led to an increase in bacteria growth, while the treatment of brewed coffee and peach tea leaves led to a decrease in bacteria growth.
- Our hypothesis was not supported because all four results were not consistent with a decrease in bacteria growth.
- The various outcomes could be due to many different factors, such as a presence of sugar in Coca-Cola and lack thereof in the three other treatments, other possible additives we didn't account for, and the caffeine content of each treatment.
- Due to the difference in results, we are unable to conclude that treating soil with various forms of caffeine all lead to either an increase or decrease in bacteria growth.
- We only saw fluorescence in one of our untreated plates, but we sent all possible inducers regardless to see what microbes we collected in our soil. The inducer was Bacillus thuringiensis.
- All our microbes were of the same genus, bacteria, all related by a common ancestor.

RESEARCH QUESTIONS:
1. How does the addition of different caffeinated compounds affect bacterial growth in soil?
2. Do our soil bacteria induce biofilm formation in B. subtilis?
3. What soil bacteria did we find?

METHODS AND MATERIALS:

FUTURE DIRECTION:
- Further research using various controls would need to be done to make this overall conclusion.
- For example, performing an experiment that includes a Coca-Cola without a sugar additive and comparing it to the regular Coca-Cola would help rule out the influence that sugar had on the result of the bacteria growth.

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