The Antimicrobial Effects of Cinnamon on the Soil's Microbiome

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AFFILIATIONS

DNA Learning Center BARCODING 101

NYC URBAN BARCODE PROJECT

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00. Abstract

Antimicrobial resistance is a growing concern as bacteria resist antibiotics, making infections difficult to treat. The development of antimicrobial resistance is a complex process involving genetic mutations and overuse and misuse of antibiotics. Can these specific substances be used as a new antibiotic against bacteria? This study aims to use DNA barcoding in determining the change in the microbiome of the soil at East Of Mccarren Park located in Brooklyn before and after treatment with Cinnamon. This would help us determine if our antimicrobial agent can be used to treat specific bacteria. In this lab, using the DNA barcoding protocols (Collecting specimens, Isolating, Amplifying, and analyzing PCR), the results identified types of possible bacteria that were grown within the cinnamon solution, suggesting cinnamon was able to suppress specific types of bacteria and enhance other types of possible bacteria.

01. Introduction

Scientists alter common compounds that could have once been dangerous to humans to provide treatment for common human illnesses. Morphine, a common painkiller, is derived from poppies. Salicylic acid, a chemical used in skincare as an acne treatment or chemical exfoliant is derived from the willow tree. (Veeresham, 2012) All over the world, medicine is created from nature around us. Even the very supplements and ingredients we have at home are used as a treatment against several illnesses. My research highlights ' The Antimicrobial Effects of Cinnamon on The Soil's Microbiome.' The significance of this study is that if it is found that these specific antimicrobial agents can decrease or increase a bacteria population, then they could potentially be used in the creation of a new antibiotic. Antimicrobial resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. Can these specific substances be used as a new antibiotic against bacteria? What bacteria are resistant to the possible antibiotic? We hypothesize that the bacteria will survive after treatment with the antimicrobial substance cinnamon, due to the richness of its compound cinnamaldehyde which ignites cell proliferation and differentiation. Current-day antibiotics are generalized as a result that bacteria that survived can easily mutate to be resistant to these antibiotics. Using soil biodiversity, we can test what specific bacteria are affected. Antimicrobial herbs such as cinnamon and antimicrobial medication are usually used to benefit patients' health or inhibit the growth of or kill certain types of bacteria in a patient. This approach offers a compassionate way to eliminate gnats indoors, as it does not result in their death. Instead, it deters them from lingering near your plants, preventing them from laying eggs in the soil and seeking more fertile environments (Slack, 2022). With the growing concern about antibiotic resistance, there is a growing need to investigate alternative antimicrobial agents, such as natural compounds like cinnamon, that may be able to mitigate this global issue... Understanding how these substances affect the soil microbiome is critical for assessing their potential environmental effects and efficacy in microbial population control. This study aims to advance our understanding of natural antimicrobial agents and their implications for soil health.

02. Objective

- To use DNA barcoding to determine the change of microbiome of the soil collected from the North/East of Mccarren Park located in Brooklyn before and after treatment with the antimicrobial agent being cinnamon and to see if it had a better resistance against bacteria or not.

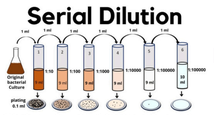
03. Problem and Hyphothsis

Antimicrobial resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. Can this specific substance be used as a new antibiotic against bacteria? What bacteria are resistant to the possible antibiotic?

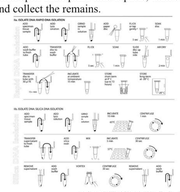
We hypothesize that the bacteria will survive after treatment with the antimicrobial substance cinnamon, due to the richness of its compound cinnamaldehyde which ignites cell proliferation and differentiation.

Procedures:

1. Collect soil samples from the North Eastern part of Mccarren Park in Brooklyn.
2. Perform hand sampling; remove small portions of soil from the ground. One sample from the park.
3. Preparation of soil; serial dilution 30 times (stepwise series of dilutions is used to estimate the concentration of a sample).
4. Use the diffusion method (the act of dispersing and determining antimicrobial activity).



5. We then cultured and intubated for two days; three control groups and three experimental groups
6. Then split each plate into 5 parts, then swob and collect the remains.



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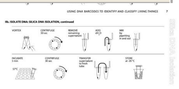
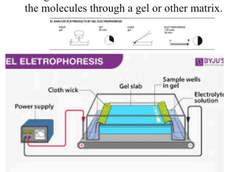
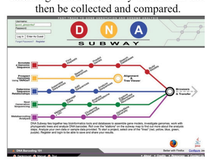


8.

9. Electrophoresis is a laboratory technique used to separate DNA, RNA, or protein molecules based on their size and electrical charge. An electric current is used to move the molecules through a gel or other matrix.



10. Then substances will be sent to sequencing throwing the DNA Subway where data will then be collected and compared.



04. Methodology

Data Interpretation Plan
Sampling:
We collected soil samples from the North Eastern part of Mccarren Park in Brooklyn on March 6th, 2024. We did this by performing hand sampling; removing small portions of soil from the ground.

Experimental question

1. **What is the effect of Cinnamon on the microbiome of soil?**
2. **What are the bacteria that resisted against the substance?**

Data to be Collected

- **Type of bacteria left and found within the soil. Frequency- (Day one)**

Method Of Measurement

1. **DNA will be extracted from the soil**
2. **DNA will be amplified using PCR**
3. **DNA will be sent out to be sequenced**

Analysis
After Sequencing:

- **Sequences will be inputted into DNA Subway to assess the microbiome of each group.**
- **The sequences will be aligned and trimmed to eliminate any part of the sequence that may skewer results.**
- **The trimmed sequence will be put through the BLAST databases to identify the microbe.**

Analysis:

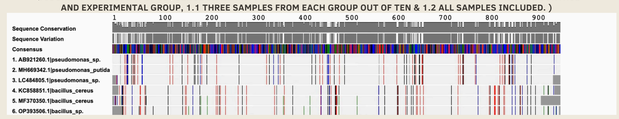
Compare bacteria present from the control and the treated soil to find out what bacteria survived after treatment.

06. Results

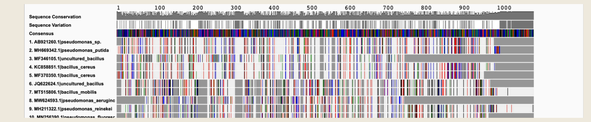
	C	1	2	3	4	5	6
C	-	92.16	92.16	91.85	91.73	90.68	90.78
1	92.16	-	100.00	99.79	83.64	82.51	82.73
2	92.16	100.00	-	99.79	83.64	82.51	82.73
3	91.85	99.79	99.79	-	83.80	82.74	82.92
4	91.73	83.64	83.64	83.80	-	99.00	98.92
5	90.68	82.51	82.51	82.74	99.00	-	99.33
6	90.78	82.73	82.73	82.92	98.92	99.33	-

	C	1	2	3	4	5	6	7	8	9	10
C	-	76.40	75.34	76.25	78.30	77.41	70.25	72.58	69.00	75.32	72.24
1	76.40	-	100.00	73.60	83.59	82.56	45.87	45.42	50.10	48.41	47.80
2	75.34	100.00	-	73.50	83.33	81.76	45.94	44.59	50.10	47.63	47.80
3	76.25	73.60	73.50	-	86.70	87.70	46.50	46.68	48.80	50.09	49.54
4	78.30	83.59	83.33	86.70	-	99.00	45.36	45.00	49.80	48.48	48.13
5	77.41	82.56	81.76	87.70	99.00	-	44.49	44.62	49.60	48.29	47.90
6	70.25	45.87	45.94	46.50	45.36	44.49	-	99.76	66.05	76.65	73.52
7	72.58	45.42	44.59	46.68	45.00	44.62	99.76	-	66.05	78.47	73.39
8	69.00	50.10	50.10	48.80	49.80	49.60	66.05	66.05	-	80.96	84.66
9	75.32	48.41	47.63	50.09	48.48	48.29	76.65	78.47	80.96	-	92.59
10	72.24	47.80	47.80	49.54	48.13	47.90	73.52	73.39	84.66	92.59	-

(FIGURE 1: THE DISPLAY OF SEQUENCE SIMILARITY PERCENTAGE BETWEEN BOTH BACTERIA DETECTED WITHIN THE CONTROL AND EXPERIMENTAL GROUP, 1.1 THREE SAMPLES FROM EACH GROUP OUT OF TEN & 1.2 ALL SAMPLES INCLUDED.)



(FIGURE 2: THE NCBI MULTIPLE SEQUENCE ALIGNMENT VIEWER (MSA) IS A GRAPHICAL DISPLAY FOR NUCLEOTIDE AND PROTEIN SEQUENCE ALIGNMENTS. AS WELL AS THEIR GENES. THREE OUT OF TEN FROM EACH GROUP ARE DISPLAYED.)

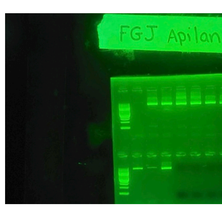


(FIGURE 3: THE NCBI MULTIPLE SEQUENCE ALIGNMENT VIEWER (MSA) IS A GRAPHICAL DISPLAY FOR NUCLEOTIDE AND PROTEIN SEQUENCE ALIGNMENTS. AS WELL AS THEIR GENES. ALL TEN SAMPLES ARE DISPLAYED.)

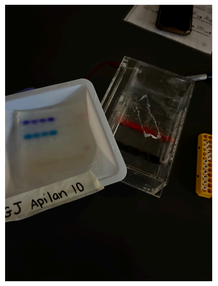
05. Analysis

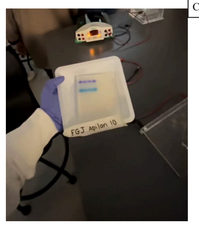
THIS TABLE SHOW THAT PSEUDOMONAS WAS DETECTED IN ALL 5 TRAILS OF EXPERIMENTAL.
AND ALL CONTROL GROUP EXCEPT TRAIL 4 DETECTED BACILLUS

Trails	Type and number of bacteria left in soil (day 1)
Cinnamon Trial 1	Pseudomonas
Cinnamon Trial 2	Pseudomonas
Cinnamon Trial 3	Pseudomonas
Cinnamon Trial 4 Cinnamon Trial 5	Pseudomonas Pseudomonas
Control Trail 1	Bacillus
Control Trail 2	Bacillus
Control Trail 3	Bacillus
Control Trail 4	N/A (unable to be detected during blast)
Control Trail 5	Bacillus



(DNA GEL ELECTROPHORESIS RESULTS: GEL ELECTROPHORESIS IS A MOLECULAR BIOLOGY METHOD USED TO ANALYZE AND SEPARATE DNA FRAGMENTS BASED ON THEIR SIZE, IN THIS IMAGE, ALL 10 OF OUR PCR MOLECULAR PHOTOCOPYING," THE POLYMERASE CHAIN REACTION", CONTAIN DNA WITH A KNOWN BARCODE GENE SEQUENCE.)

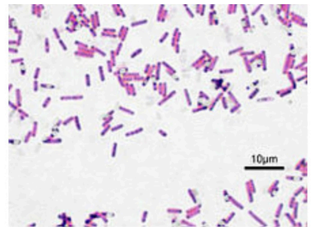




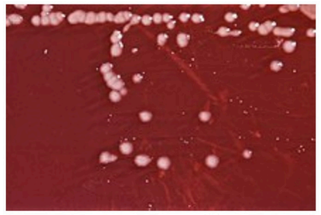
THIS IMAGIE IS REPRESENTING THE GELL AFTER ELECTROPHROSIS PROCESS

Materials:
Gloves Tweezers Ziploc Bags/containers Freezer
Microscope Water Soil Samples Petri dishes Marker Heat Block Test Tubes Pipettor
Racks Tips Discard Bucket

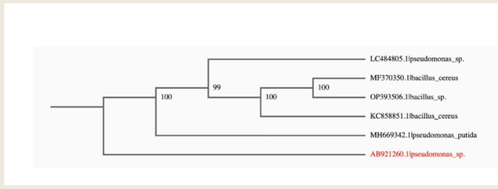
BACILLUS (FOUND IN EXPERIMENTAL, CINNAMON-TREATED SOIL)
BACILLUS IS A GENUS OF GRAM-POSITIVE, ROD-SHAPED BACTERIA KNOWN FOR THEIR ABILITY TO FORM ENDOSPORES, WHICH ALLOW THEM TO SURVIVE IN HARSH ENVIRONMENTAL CONDITIONS. THEY ARE WIDELY DISTRIBUTED IN NATURE AND ARE FOUND IN VARIOUS HABITATS.



PSEUDOMONAS (FOUND IN CONTROL)
PSEUDOMONAS IS A GENUS OF GRAM-NEGATIVE, ROD-SHAPED BACTERIA KNOWN FOR THEIR METABOLIC DIVERSITY AND ENVIRONMENTAL ADAPTABILITY. MEMBERS OF THIS GENUS ARE COMMONLY FOUND IN VARIOUS NATURAL AND ARTIFICIAL ENVIRONMENTS, WHERE THEY PLAY CRUCIAL ROLES IN ECOLOGICAL PROCESSES, INCLUDING NUTRIENT CYCLING AND BIODEGRADATION.



WITHIN EACH GENUS, THE SPECIES SHARE A HIGH GENETIC SIMILARITY, INDICATING HOW MUCH THEY ARE CLOSELY OR DIFFERENTLY RELATED. (DISPLAYED IN THE FOLLOWING FIGURES 1, 2 & 3).



(FIGURE 4: PHYLOGENETIC ANALYSIS PROVIDES AN IN-DEPTH UNDERSTANDING OF HOW SPECIES EVOLVE THROUGH GENETIC CHANGES. THE TREE SEPARATES PSEUDOMONAS SPECIES FROM BACILLUS SPECIES, INDICATING DISTINCT EVOLUTIONARY LINEAGES. THIS SEPARATION IS CONSISTENT WITH THEIR CLASSIFICATION INTO DIFFERENT GENERA.)

07. Disscusion and Conclusion

Cinnamon has properties including cinnamaldehyde and eugenol which are known to possess antimicrobial properties. The fact that there were no Pseudomonas in the cinnamon-treated soil indicates that these compounds effectively inhibit the growth of Pseudomonas, either by killing them or by preventing their growth. This means that cinnamon's antimicrobial properties selectively suppress the growth of Pseudomonas while promoting Bacillus survival. From Figures 1 and 2 these groups may likely be affected differently by the antimicrobial compounds contained in cinnamon due to possible genetic differences between Pseudomonas and Bacillus groups. Given a mere 20% genetic difference (Figure 2), it is suggested that cinnamon's antimicrobiotic characteristics hinder Pseudomonas more than Bacillus possibly due to variations in their DNA profile as supported by our data. The phylogenetic analysis supports the idea that these two genera are distinctly different and may respond differently to antimicrobial agents like those found in cinnamon (Figure 4). In summary, the combined results from microbial presence analysis, phylogenetic tree construction, and multiple sequence alignment reveal that Bacillus and Pseudomonas species exhibit distinct genetic and metabolic traits that influence their responses to environmental factors such as antimicrobial compounds in cinnamon. Bacillus species' presence in cinnamon-treated soil and their high genetic similarity suggest that they possess adaptive mechanisms to survive or benefit from cinnamon's compounds. In contrast, the absence of Pseudomonas in the same environment indicates a susceptibility to cinnamon, likely due to the lack of necessary adaptations.

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09. Acknowledgements

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