The Effects Of Antibacterial Agents On The Biodiversity Of Bacteria In The Soil

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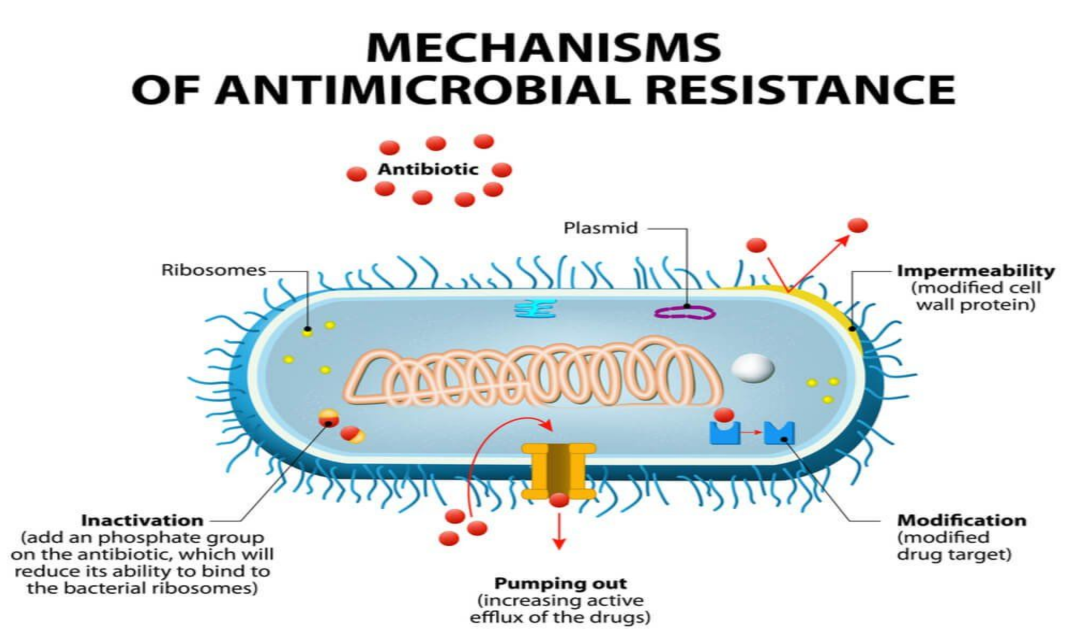
ABSTRACT

The study attempted to investigate the change in the diversity of bacteria after the soil taken from our backyards are treated with plant extracts, known to contain antimicrobial properties. After the serial dilution of the plant extracts and soil samples, DNA was extracted from the soil samples in the barcoding center. The same DNA samples of soil samples were to be amplified using PCR techniques. The soil samples were sent out to be sequenced.



INTRODUCTION

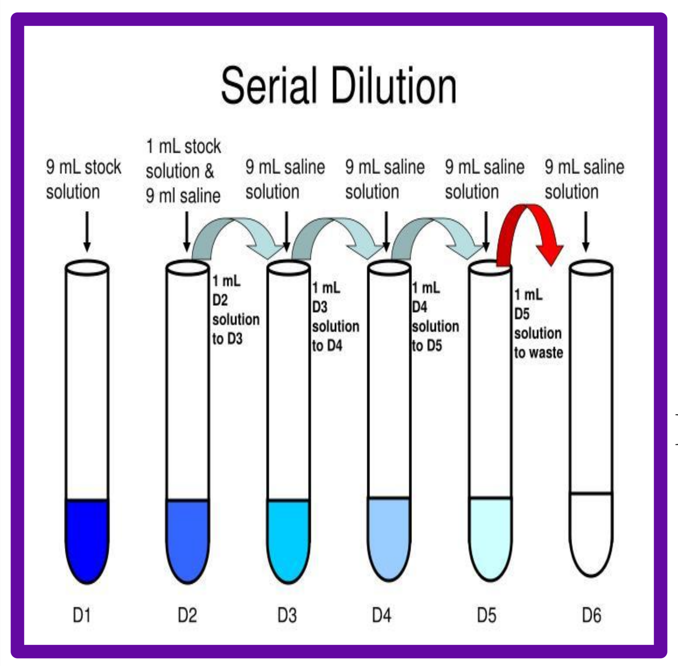
In this project, the soil samples in the backyard of two different locations were collected. The soil samples came from two different neighborhood boroughs of New York City: Queens and Brooklyn. Each of the members obtained two soil samples from each location. The antibiotics were obtained from antimicrobial plants in order for us to determine an antibacterial agent or an antibiotic that can kill specific bacteria found in soil. The types of plants that were used in the experiment also contained secondary metabolites were basil, dill, garlic, and onions, which have been known to have antimicrobial properties.

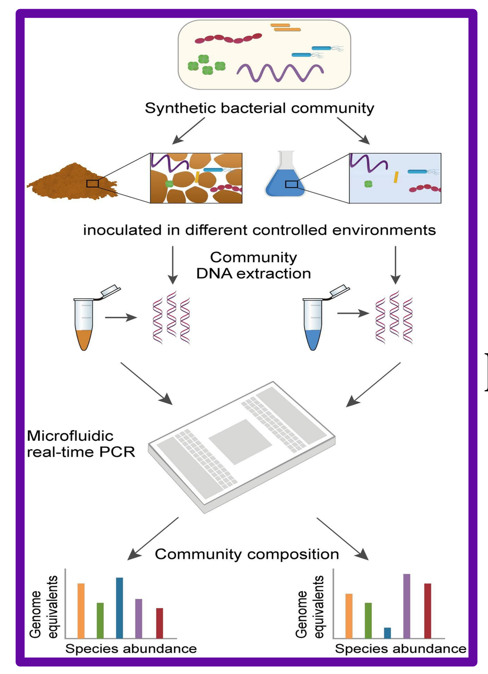
MATERIAL

METHODOLOGY

- Soil, Basil, 20 test tubs, 2 small jars, Saline solutions, Distilled water, pasteur pipette, agar petri dishes, Cotton swabs, Inoculating Loops, Small specimen containers

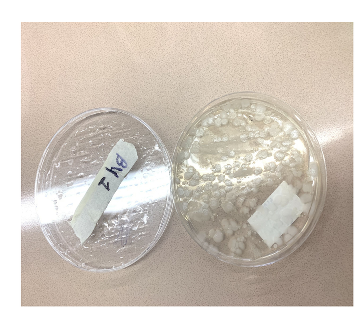
Process of Serial dilution for soil sample and plant extract





PCR process

Bacteria grew on the agar dish



DATA

Mixture: Plant Extract #__ + Soil Sample #__	Date Collected	Location of Sample soil collected	The temperature of the day of Collection	The discovered Bacterial colonies (Dill)	The discovered Bacterial colonies (Garlic)	The discovered Bacterial colonies (Basil)
Without Plant Extract + Soil Sample #10 (Control)	February 23, 2020	Q336+MG Queens, New York (40.7541820, -73.9387370)	45°F	• *Bacillus Cereus* • *Bacillus thuringiensis* • *Bacillus paramycoides*	• *Bacillus Cereus* • *Bacillus tropicus* • *Bacillus sp.* • *Bacillus thuringiensis*	• *Bacillus* • *Bacillaceae* • *Glacial ice bacterium*
Plant Extract #1 + Soil Sample #10 (Experimental)	February 23, 2020	Q336+MG Queens, New York (40.7541820, -73.9387370)	45°F	• *Dyella japonica* • *Dyella terrae* • *Uncultured marine bacterium*	• *Uncultured bacterium* • *Uncultured proteobacterium* • *Raisionia pickettii*	• *paenibacillus favisporus* • *Lysinibacillus*
Plant Extract #5 + Soil Sample #10 (Experimental)	February 23, 2020	Q336+MG Queens, New York (40.7541820, -73.9387370)	45°F	• *Dyella jiangningensis* • *Dyella terrae* • *Dyella japonica*	• *Raisionia pickettii* • *Uncultured Raistonia sp.* • *Uncultured bacterium* • *Uncultured proteobacterium*	• *Bacillaceae* • *paenibacillus* • *paenibacillus cineris*

RESULTS!

Discussion

Based on the treatment of the soil samples it can be concluded that the antimicrobial agents did have an effect on the growth of the bacteria.The controlled agar plate with no treatment had a significant number of bacterial growth while the 5th and 10th diluted sample agar plate had significantly less bacterial growth due to the antimicrobial treatment. The data table below shows the antimicrobial agent Anethum graveolens (dill), Ocimum (basil) and garlic can alter the growth of bacteria and its diversity of the soil.

Moreover, the use of the Basic Local Alignment Search Tool (BLAST) to identify sequences in databases also helped us identify the type of most common bacteria present in our diluted samples. It also helped us conclude that the antimicrobial agents have no significant effect on the growth of soil microbiomes. However, it was notable that some of the frequent bacteria present in the samples mutated after its treatment with the plant extracts. For example, bacteria such as Bacillus Cereus, Bacillus thuringiensis, Bacillus paramycoides in the first undiluted sample most likely mutated to Dyella japonica, Dyella terrae, and Uncultured marine bacterium.

recommendation

For future experiments, the concentration of the antimicrobial agents used during serial dilution in order to prevent more bacterial colonies' growth can be increased. Another type of antimicrobial agents such as herbs that are proven to have antimicrobial properties such as herbal teas like oolong teas, oregano, and mint teas could also be used. Instead of 1 gram of the antimicrobial sample, the concentration could also be changed to 5 grams, however, the 5-fold serial dilution technique will still be used. This will hopefully decrease the amount of colonies growth on treated soil samples.

Reference

1. The US National Library of Medicine National Institutes of Health Search database. (2019). Expanding the soil antibiotic resistor: exploring environmental diversity. (p. https://www.ncbi.nlm.nih.gov/pubmed/17951101). PubMed.

2. Stormwater runoff carries antibiotic-resistance to urban beaches. (2019). Retrieved 13 November 2019, from https://www.stormh2o.com/bmps/article/21107891/stormwater-runoff-carries-antibioticresistance-to-urban-beaches

3.Principal methods for isolation and identification of soil microbial communities. (2019). Retrieved 13 November 2019, from https://www.ncbi.nlm.nih.gov/pubmed/22791233

4. Polymerase Chain Reaction (PCR) Fact Sheet. (2015, June 16). Retrieved November 13, 2019, from https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet.

5. M.Claire Horner-Devine1*, KarenM.Carney2 andBrendanJ.M.Bohannan (2003). "An ecological perspective on bacterial biodiversity". Retrieve in October 2019. Available URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1691570/pdf/15058386.pdf

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