A Pilot Research Project to Enhance Inquiry-Based Learning by Mapping The Microbiome of the Southern Appalachian Region

Shivam Patel, Sean Fox
Department of Health Sciences, **East Tennessee State University**

Abstract

As humans continue to advance healthcare resources, we face a growing threat of nosocomial multidrug-resistant bacteria. The rise of these antibiotic-resistant microorganisms has been placed on the World Health Organization's watchlist as one of the biggest threats to global health. We continue to have a shortage of effective antibiotics with the rise of these "superbugs". With the growing number of deadly pathogens, the future of medicine relies on scientific findings to combat multidrug-resistant bacteria. Appalachia could be the answer to combat this new health threat. As the most biodiverse temperate forest region in North America, our beautiful backyard in the Smoky Mountains contains a plethora of microorganisms that have become genetically diversified over billions of years. Many of these soil bacteria naturally produce their own antibiotics. With the wide variation of natural bacteria, Appalachia serves as a testing ground to harness the power of natural antibiotics. A gram of soil contains more than 10,000 different species of bacteria. The biodiversity of these microbes is largely unknown, as almost 99% of these species cannot be cultured in a normal lab setting. This pilot project will lay the foundations of discovering Appalachia's microbiota which has, thus far, never been cataloged.

Background

This pilot project encompasses traveling across the southern Appalachian region to collect soil samples, developing techniques to grow these unique microbes, as well as conducting biological assays of soil microbes to test for potential novel microbial interactions and antimicrobial properties with priority of discovering new treatments for human pathogens.

The purpose of this novel project is to find optimal growth conditions for soil bacteria in a laboratory setting. It will serve as a pilot study to eventually compare the difference between lab-grown cultures and the true diversity of microbes in soil through direct soil DNA extraction and sequencing.

After culturing soil bacteria, samples will be further analyzed in the laboratory to attempt to determine their diversity (microbial fingerprint) through 16S ribosomal RNA sequencing and tested for the presence of novel antimicrobial compounds. The main goals of this study are to 1) Characterize & catalogue the microbiome of the southern Appalachian Mountains; 2) Characterize the interactions of the microbiome in this unique region.

Methods

Topsoil was collected on different elevations and locations on Buffalo Mountain located in Johnson City, TN. Samples were then processed in the laboratory. First, 1 gram of soil was taken from each stock soil and put in individual test tubes. The original soil stocks were volumized to 50mL with phosphate-buffered solution (PBS) and vortexed to homogenize the mixture, then filtered to collect liquid nutrients excluding the bacteria.

Filtered liquid nutrient from their respective tubes were mixed with nutrient agar in 1:10 ratios to create petri plates that are 90% agar and 10% soil nutrient.

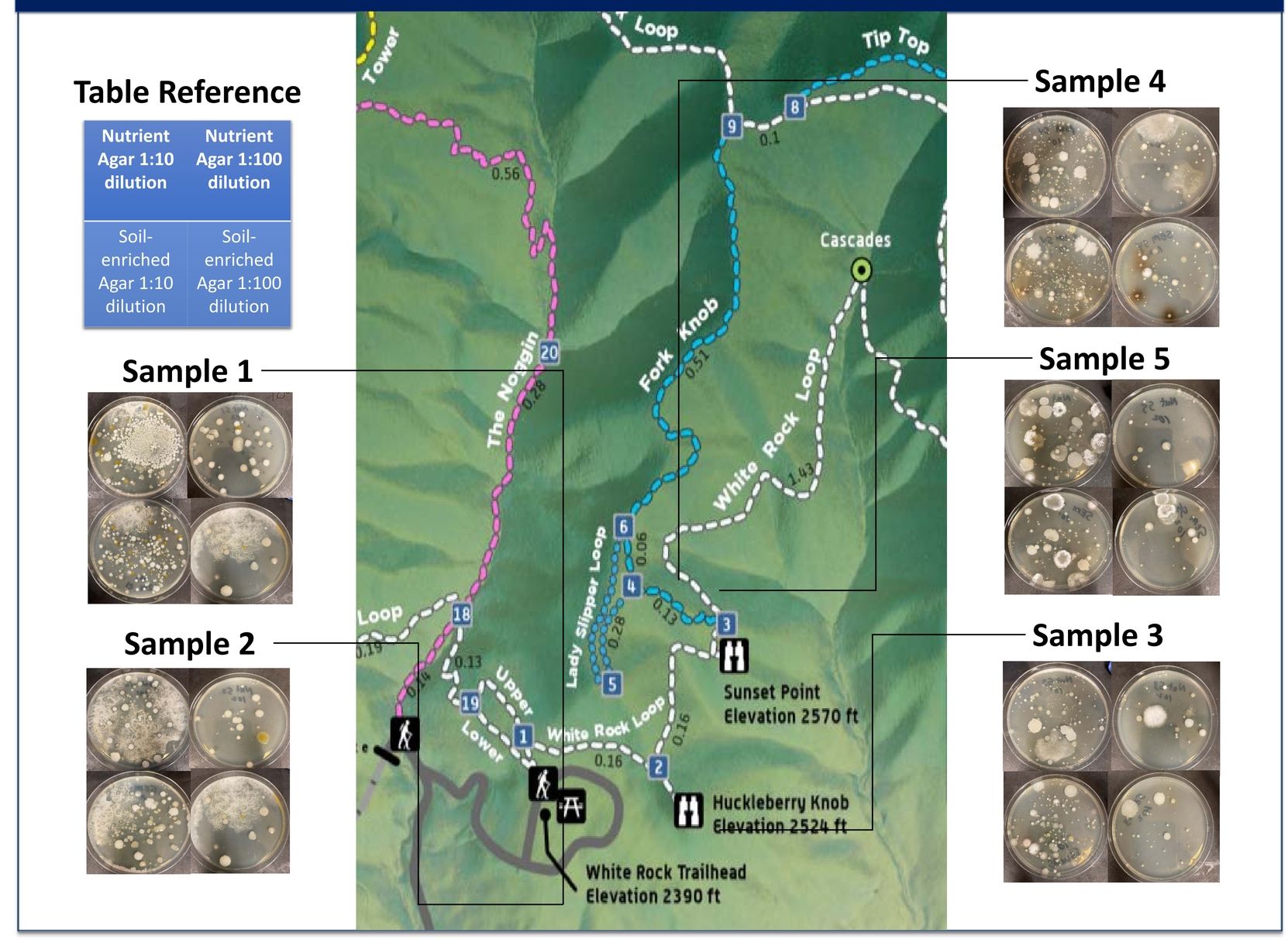
To grow the soil microbes, 25mL of PBS was added to each 1g of soil and vortexed. After letting the soil settle, 100µL of the soil slurry was taken and plated on nutrient agar and 10% soil-enriched nutrient agar. These conditions allow the comparison of growth and whether infusing petri plates with native nutrients increased the diversity of laboratory-grown cultures.

Cultures were monitored over the next week to observe growth, diversity, and microbial interactions. Colonies were counted, and 2 colonies from each plate were used for Polymerase Chain Reacted (PCR) with 16S primers (Total volume=25 µl). 7 µl PCR products were screened via gel electrophoresis and the rest of the sample processed for Sangar Sequencing.

Result – Figure 1

Table Reference

Nutrient Agar 1:10 dilution	Nutrient Agar 1:100 dilution
Soil-enriched Agar 1:10 dilution	Soil-enriched Agar 1:100 dilution



Results – Figure 2

Flavobacterium sp. HP3M 16S ribosomal RNA gene, partial sequence
Sequence ID: KM187454.1 Length: 1379 Number of Matches: 1

Range 1: 161 to 667 GenBank Graphics Next Match

Score	Expect	Identities	Gaps	Strand
876 bits(474)	0.0	503/516(97%)	9/516(1%)	Plus/Plus

```
Query  184  AAAGTATAGAGCATGCGTCCTCATTAGTCTAGTCTGGTAAGGTAACGGCATACCAAGGCA  243
Sbjct  161  AAAG-AT-GAGCATGCGTCC-CATTAG-CTAGT-TGGTAAGGTAACGGCTTACCAAGGCA  215
Query  244  ACGTATGGGTAGGGGTCCTGAGAGGGAGATCCCCCACACTGGTACTGAGACACGGACCAG  303
Sbjct  216  ACG-ATGGGTAGGGGTCCTGAGAGGGAGATCCCCCACACTGGTACTGAGACACGGACCAG  274
Query  304  ACTCCTACGGGAGGCAGCAGTGAGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCA  363
Sbjct  275  ACTCCTACGGGAGGCAGCAGTGAGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCA  334
Query  364  TGCCGCGTGCAGGATGACGGTCCTATGGATTGTAAACTGCTTTTGTACGAGAAGAAACAC  423
Sbjct  335  TGCCGCGTGCAGGATGACGGTCCTATGGATTGTAAACTGCTTTTGTACGAGAAGAAACAC  394
Query  424  TCCTATGTATAGGAGCTTGACGGTATCGTAAGAATAAGGATCGGCTAACTCCGTGCCAGC  483
Sbjct  395  TCCTATGTATAGGAGCTTGACGGTATCGTAAGAATAAGGATCGGCTAACTCCGTGCCAGC  454
Query  484  AGCCGCGGTAATACGGAGGATCCAAGCGTTATCCGGAATCATTGGGTTTAAAGGGTCCGT  543
Sbjct  455  AGCCGCGGTAATACGGAGGATCCAAGCGTTATCCGGAATCATTGGGTTTAAAGGGTCCGT  514
Query  544  AAGCGGTTTAGTAAGTCAGTGGTGAAAGCCCATCGCTCAACGGTGGAACGGCCATNGATA  603
Sbjct  515  AGGCGGTTTAGTAAGTCAGTGGTGAAAGCCCATCGCTCAACGGTGGAACGGCCATTGATA  574
Query  604  CTGCTGAACTTGAATTATTAGGAAGTAACTAGAATATGTAGTGTAGCGGTGAAATGCTTA  663
Sbjct  575  CTGCTGAACTTGAATTATTAGGAAGTAACTAGAATATGTAGTGTAGCGGTGAAATGCTTA  634
Query  664  GAGATTACATGGGAATACCAANTNGCGAAGGGCAGG  699
Sbjct  635  GAGATTACATGG-AATACCAATT-GCGAAGG-CAGG  667
```

Representative BLAST alignment. from the National Center for Biotechnology Information's database, indicates the genus of this bacteria is Flavobacterium. The16S region of 20 samples were amplified by PCR and successful samples were sent in for Sanger Sequencing.

Results – Figure 3

	Coordinates	Nutrient Agar Colony Count	Soil-Infused Agar Colony Count	Sequenced Bacteria Data
S1	Height: 2271 ft, 36.278, -82.345	4.7×10^3	6.7×10^3	Pedobacter. Flavobacteriuam. 1 unrecognized genus
S2	Height: 2271, 36.278, -82.345	2.5×1.0^3	1.5×10^3	Flavobacterium
S3	Height 2520, 36.277 -82.352	3.5×10^3	3.8×10^3	Paraburkholderia, Chitinophaga
S4	Height: 2707 ft, 36.176, -82.349	7.2×10^3	9.2×10^3	Streptomyces, 1 unrecognized genus
S5	Height: 2707 ft, 36.176, -82.349	2.8×10^3	3.2×10^3	Viridibacillus Paenibacillus Burkholderia

Conclusion

- When culturing soil microbes, Soil Enriched Media (SEM) grew microbes in both greater numbers of microbes and the diversity of microbes present vs standard nutrient agar media
- The populations from each of the 5 sources of soil from Buffalo Mountain produced unique microbial populations and profiles specific to their location.
- 16S amplification by PCR directly from bacterial colonies grown on SEM proved to be a successful and efficient method as high quality DNA amplification was produced in both quantity and quality.
- Sequencing data in Figure 2 and 3 show that 16S sequencing allows a qualitative understanding of soil samples.

Acknowledgements

- Special thank you to the East Tennessee State University Honor's College for supplying the resources needed for this presentation
- Thank you to Johnson City Parks and Recreation for permission to collect sample from Buffalo Mountain.
- Thank you to the ETSU Molecular Biology Core Facility for sequencing service