Analyzing the Effects of Roadside Pollution on Garden City Lichen Species through DNA Barcoding

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Abstract

Lichens exhibit a **mutualistic** relationship between a **fungus** and a photosynthetic organism, usually **algae**. They are sensitive to many pollutants, such as **sulfur dioxide** and **nitrogen dioxide**, which are often produced by **automobiles**. This experiment aims to address the **correlation** between various lichen species, the **distance** that each grows from the road, and whether roadside pollutants have an effect on what **type** of lichen species grows in which location. The Lichen DNA was purified and amplified using a **PCR**. These results were analyzed using gel electrophoresis and were further analyzed on various bioinformatics platforms such as **DNA Subway** and **Gear Genomics**.

Introduction

Lichens are **indicator species** known for their sensitivity to air pollution. Lichen are especially susceptible to compounds like SO_2 and NO_2, both of which are emitted by vehicles. The three types of lichen have different sensitivities to these pollutants but an **excess** of these compounds can be **fatal to all**. Previous studies have found that crustose is least sensitive and fruticose is most sensitive, leading us to believe that there is a large disparity in the type of lichen **near roads**.

This project aims to use **DNA Barcoding**, a process which can be used to sort and identify species using segments of DNA, to identify lichen and compare the abundance of test species based on their proximity to the road. Pictures will be taken of each sample and location coordinates will be collected.

Research Question

Are lichens good indicators of high concentrations of roadside pollutants?

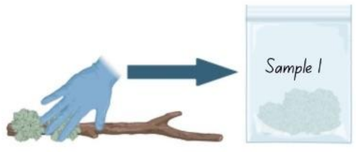
Hypothesis

There will be **fewer** lichen closer to **roads** and well inhabited areas compared to more isolated stretches of land.

Explanation: Because lichen are sensitive to pollution, we expect that there will be fewer lichen that exist in close proximity to the roads.

Goals

Process	Details
Collecting Samples	• Take pictures of each sample • Track coordinates of each location • Place each sample in a labeled bag • Measure distance from road • Count number of lichen at each location
Extracting DNA, PCR Amplification, Electrophoresis, and Barcoding	• Sort each lichen by sample • Determine species of lichen (only for species with successful PCR analyzed via gel electrophoresis)
Analysis	• Sort each lichen by distance from the road • Compare populations at each distance • Compare species/type at each distance

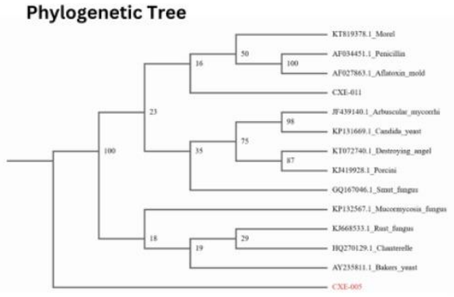


Methodology

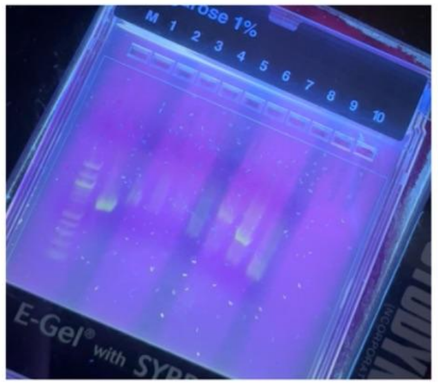
Collection of Samples	• Samples will be collected from around Garden City as well as in the surrounding area once permission is granted, gloves will be worn to collect samples to avoid contamination • Approximately 20 samples of lichen will be collected, each sample being placed in its own separate bag labeled with a number
Extracting/Purifying DNA	• Use lysis buffer and a miniature pestle to break down the cells • Use Silica Resin to bind to the DNA • Clean and purify the DNA using wash buffer, deionized water, and a centrifuge • Use Chilled Ethanol to rid the DNA of water molecules • SAFETY: Hair will be tied back, gloves and goggles will be worn
Amplification of DNA via PCR	• Amplify the amount of DNA for each lichen sample in order to have enough samples of DNA so that barcoding can be analyzed accurately
Gel Electrophoresis	• Using agarose gel electrophoresis, the amplified DNA fragments will be separated by size, the smallest at the bottom of the tray • Indicates if the PCR was successful so barcoding can be completed
Barcoding	• Will be used to identify the species collected within Garden City
Analyzing data	• Use DNA Subway/Genomics to analyze barcoding results
Conclusion	• Determine whether proximity to the road affects the growth and species types of lichen

Results:

Phylogenetic Tree

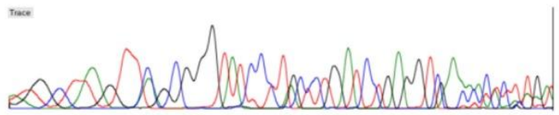


Gel Electrophoresis (CXE-005 to CXE-012)

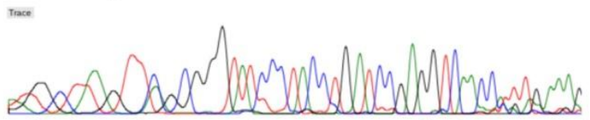


Results (cont.)

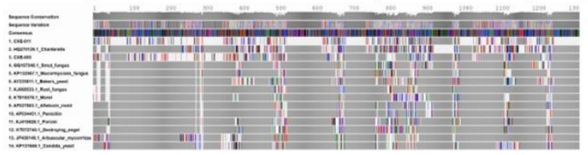
Reverse Sequence Abundance of CXE-005



Reverse Sequence Abundance of CXE-011



Muscle Diagram (CXE-011 and CXE-005 compared to known species)



Reverse of CXE-011 to Physcia Millegrana

Score	Expect	Identities	Gaps	Strand
852 bits(461)	0.0	483/494(98%)	2/494(0%)	Plus/Minus

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Query  23   GTCAACCTTTGAAAGTGTACGGTTGTTTGGCCGGCACACANTGAGAGGCTTGCAAAGCGG  82
Sbjct  492  GTC-ACCATTG-AAGTGTACGGTTGTTTGGCCGGCACACAGTGAGAGCCTTGCAAAGCGG  435
Query  83   GATGTCAAAATCTTGCTACGCTTCAAGTCTCGCTGGACCGCCACGAAATTGCGGGCACGT  142
Sbjct  434  GATGTCAAAATCTTGCTACGCTTCAAGTCTCGCTGGACCGCCACGAAGTTGCGGGCACGT  375
```

Forward of CXE-005 to Hyalorbilia Erythrostigma

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Score:647 bits(350), Expect:0.0,
Identities:441/484(91%),  Gaps:15/484(3%), Strand: Plus/Plus

Query  99   CCATCTCTTGTGAAAC-ACCCCTTTGCTTTGGTGGC---TGTCCGCCTC-TCGCAGGCAG  153
Sbjct  283  CCA-CTCTTGTGAAACTACCCCTTTGCCTTGGTGGCCGCAGTTTGCCTCGCCGCTGACAG  341
Query  154  CCGTAAGTCCACCAGTCAAACTCTTCAAAAACCTTTAGTCTGAACAAAACATTCATAAAG  213
Sbjct  342  -CGTAAGTCCGCCAGCTAAACTCTTCAGAAACCTTTAGTCTGAACAAA--ATTCATAAAG  398
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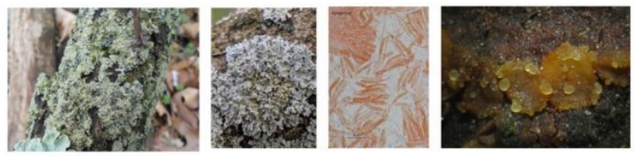
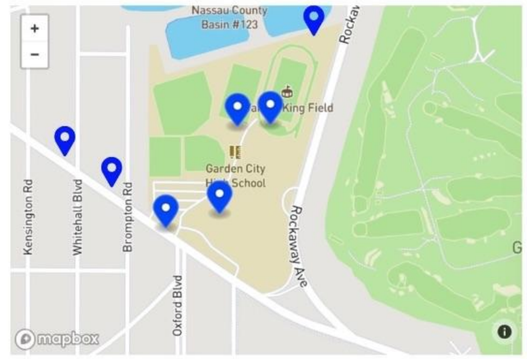


Physcia Millegrana **Hyalorbilia Erythrostigma**

Locations and Samples



Sample #	Coordinates	Sample #	Coordinates
1	40.734°N 73.653°W	5	40.735°N 73.665°W
2	40.734°N 73.652°W	6	40.735°N 73.664°W
3	40.732°N 73.654°W	7	40.733°N 73.657°W
4	40.732°N 73.653°W	8	40.732°N 73.655°W

Discussion/Conclusion

Unlike the initial hypothesis, greater quantities of lichen tend to exist on trees near roads rather than in secluded areas. The BLAST results for CXE-005 (Sample #1), one of the lichens sampled far from the road, indicated that the species was most likely not a lichen, but Hyalorbilia erythrostigma, a different type of fungi which suppresses nematode populations. Nematodes are a type of parasite that can harm trees by feeding on their roots and potentially transmitting diseases. This fungus feeds on nematodes in their early stages of life, controlling the population and helping to preserve the tree. Therefore, it can be said that Hyalorbilia erythrostigma has a mutualistic relationship with trees.

CXE-011, sample #7, was the species identified as a lichen--Physcia Millegrana. This species of lichen is a type of foliose lichen, thriving in areas where pollution levels are elevated. As this lichen is tolerant to higher amounts of pollution, large populations of Physcia Millegrana can be an indicator of poorer air quality. Since there was a decent amount of lichen on the tree from which it was collected, it can be inferred that the area had higher levels of pollutants.

References

All figures are student generated using gear genomics and BioRender