How lichen microenvironment and sunlight effect biodiversity

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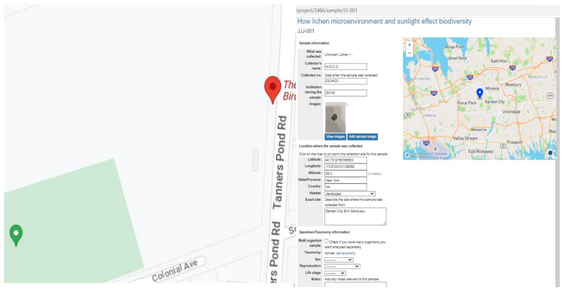
Abstract

Lichen is a group of fungus and bacteria that form a culture together making a hybrid organism. Many lichen species are still unknown and undiscovered because of the little information available about lichen. The unknown factors makes it more interesting to see how different environmental factors affect the typing of these organisms we researched. The effects of micro environmental factors on the specific types of lichen native to Long Island and we hypothesized that a darker lichen is more likely to grow in areas with little sunlight in an environment compared to lichen exposed to light have a lighter color. We collected our samples of lichen exposed to different light levels in the Garden City Bird Sanctuary to keep the environment as a constant we then started the important process of barcoding our lichen samples with a PCR reaction specific to fungi (ITS-1) we used lysis buffer to to break down the samples and used silica to extract pure DNA. Then we proceeded to Barcode our DNA until we got our final sequences.

Introduction

Our group tried to prove the effects of micro environmental factors on species in the environment, we mainly focused on light exposure in our experiment based on the peer reviewed article of lichens on road signs (see image below), which showed promise On the basis of the experiment where lichen was growing on two sides of a stop sign, one side had a larger exposure to light then the other which greatly affected the results. The published papers showed how there was a physical change in the lichen but didn't prove that these two organisms were the same or different species. Which is what my group is trying to prove.

https://www.newscientist.com/lastword/mg25433841-300-why-are-the-lichens-distributed-in-this-way-on-this-sign-pictured/



Collection Sites near Tanneŕs Pond Road and within the Bird Sanctuary

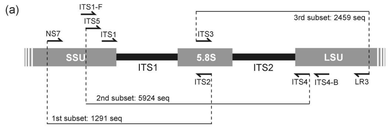
Materials & Methods:

- 50% Silica Solution (2 tubes of 100 µL)
- 2 Sterile plastic pestles
- 6 sterile toothpicks or pipette tips

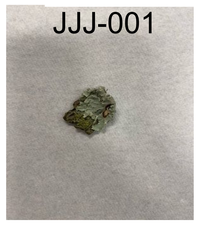
Tissue samples

- 2 microcentrifuge tubes (1.5 mL)
- 2 Microcentrifuge tube locks
- For sample storage: 95%+ EtOH [Ethanol] (2 tubes of 1000 µL) or freezer

First, add the specimen tissue sample, next add the lysis solution. Then grind the sample in the solution, and incubate for 10 minutes at 65 degrees Celsius. After incubating, centrifuge for 1 minute. After centrifuged, transfer the supernatant to a fresh tube and add silica resin. Mix them together and incubate for 5 mins at 57 degrees Celsius. Centrifuge for 30 seconds, and remove the supernatant. Then add the wash buffer, and vortex. Then repeat by adding the wash buffer, vortexing, and centrifuging for 30 seconds. Remove the remaining supernatant, and add dH20. Mix this by pipetting in and out, and incubate for 5 minutes at 57 degrees Celsius. Lastly, centrifuge for 30 seconds, transfer the supernatant to a fresh tube, and store at -20 degrees Celsius.



https://bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-10-189



pBR322/BstNI. This plasmid-based DNA ladder has fragments of 1,857; 1,058; 929; 383; and 121 bp. The ITS-1 barcode band is between 929 and 383 bp, within the expected size.

No PCR Product

Results:

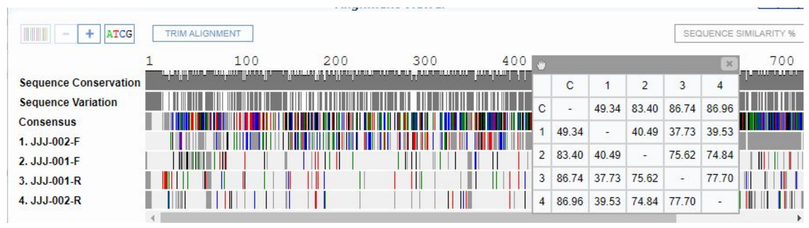
Our results were inconclusive as our barcoded DNA only had 80 % identity to other organisms and were low quality sequences. Some of our sequences clearly matched incorrect species such as stalked mushrooms, showing how ITS-1 sequences have been conserved evolutionarily across fungi species. When we proceeded to Barcode our DNA until we got our final sequences which were (below):

Tables & Figures:

Sequence Name	Forward Sequence	Organism	Reverse Sequence	
JJJ-001	CCGCTTATTGGTANGCTTAAATTCAGCGGGTAGNCNGGCCTGATTTGAGGTCCAGTTGGAAATGATGGTCTCTTGAGAGACAGTTGGAATCAGGTCCCTATAGGTTCACTAAGCCAAGGCCGCAAAGGGCTGCCCAAATGTATCNAGAGAGATCAGATCAGCCAGGGACCTGCNAGCTCCAAAATCCCANCCCAATGGCTTCCCAAAAAGATATCCGGNGGAGGAGTTCACGACGCTCGGACAGGCGTGCCCCNCGGAATGCCTAGGGGGNCANGGTGCGTTGAAAGATTCGGTGATTCACTNNGCANTTCACATTACTTATCGNATTTCNCTGCNTTCTTCGCCGATGCNAGAGCCAAGAGAACGGTTGNTGAAACTTA	Poor quality	GGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTGTCCGTCCTGATTTGAGGTCAAGTTGAAATGACTGTCTCTTGCNAGACGGTTGGAAGCAAGTCCCTATAGGTTCGCTAAGCCAAGGCNCAGATAATTATCACACCAAGGCCGCAAAGGGCTGCCCAAATGGTATTCAAGGATAGCAGATCAACCAGGGACCTGCAAGCTCCCAAATCCCACCCAATGCCTTCCNAAAAAGATATAGGGTGGAGGAGTTCACGACACTCAAACAGGCNTGCCCCNCGGAATGCCTAGGGGGCCAAGGTGCGTTCAAAGATTCGGTGATTCACTGAATTCNGCAATTCACATTACTTATCGCATTTNGCTGCNTTCTTCATCNATCCNAGAGCCAAGAGAACGGTTGNTGAAACTTA	**Agaricomycetes sp. genotype (93%) obviously not because this is a stalked mushroom, showing how poor quality sequence can yield incorrect results**
JJJ-002			TTCAGCGGGTAGTCNCGCCTGATTTGAGGTCAAGTTNGAACTGATTGCCTCTTGCAAGACGGTTGGAAGCTAAGTTATCACACCAAGGCCGCAAAGGGCTGCCCAAATGGTTCGCTAAGCCNAGGCGCAGATAATTATCACACCAAGGCCGCAAAGGGCTGCCCAAATGGTATTCAAGGATAGCAGATCAACCAGGGACCTGCAAGCTCCCAAATCCCACCCAATGCCTTCCNAAAAAGATATAGGGTGGAGGAGTTCACGACACTCAAACAGGCNTGCCCCNCGGAATGCCTAGGGGGCCAAGGTGCGTTCAAAGATTCGGTGATTCACTGAATTCNGCAATTCACATTACTTATCGCATTTNGCTGCNTTCTTCATCNATCCNAGAGCCAAGAGAACGGTTGNTGAAACTTA		

Although our sequence is low quality, one of our sequences showed the stalked mushroom, which is closely related to species of lichen. We only had two samples of DNA that showed up on the gel, because the third one wasn't a good DNA extraction or had few fungi species..

	C	1	2	3	4
C	-	49.34	83.40	86.74	86.96
1	49.34	-	40.49	37.73	39.53
2	83.40	40.49	-	75.62	74.84
3	86.74	37.73	75.62	-	77.70
4	86.96	39.53	74.84	77.70	-

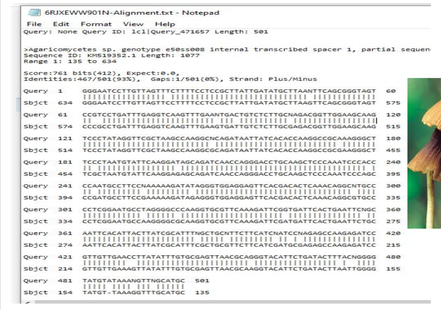


	C	1	2	3	4
C	-	49.34	83.40	86.74	86.96
1	49.34	-	40.49	37.73	39.53
2	83.40	40.49	-	75.62	74.84
3	86.74	37.73	75.62	-	77.70
4	86.96	39.53	74.84	77.70	-

Discussion

Our hypothesis was inconclusive about the effect of micro environmental factors on the species in the environment. Other articles and experiments in the scientific community along with some peer reviewed articles help the world have a broader understanding of a largely unknown species We wish we could have had a more purified DNA template in the future, If given the opportunity to continue with this research, we would like do this experiment over a larger area with many subtopics of research instead of focusing in on one.

The ITS-1 primer yielded only poor quality sequences. Although the sequences belonged to different species of fungi with about 86%-93% identity, they showed obvious errors. For example, Agaricomycetes was identified by BLAST (shown below), but since it is a stalked mushroom, it could not be our sample collected.



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Lichen this sign (Ebsco)
Why are the lichens distributed in this way on this road sign? The surfaces have the same texture. "Lichen This Sign." *New Scientist*, Reed Business Information, 29 Apr. 2022, https://www.sciencedirect.com/science/article/abs/pii/S0262407922007692.

Acknowledgements

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