



Investigating Lichen as a Bioindicator

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Abstract

In this Urban Barcoding Project, we investigated the effects of environmental pollution and climate change on lichen through comparing the DNA of the species in Central Park and Black Rock Forest. We hypothesized that if lichen species were exposed to greater levels of pollution, like in Central Park, then the lichens with the traits most fit to tolerate atmospheric pollution will be more prevalent in that area than one exposed to less pollution, like Black Rock Forest. We make this assumption because of the fact that lichen obtain all of their nutrients from the surrounding air and rain. We used the collection kit provided by the Urban Barcoding project to examine DNA sequences of 15 samples from the two locations. Unfortunately, we only had one result, so we were not approved for sequencing.

Introduction

New York City's high level of industrialization has rapidly increased the emission of greenhouse gasses into the atmosphere. Changing temperatures, coupled with numerous other effects of climate change, alter plant and wildlife populations' diversity in New York's ecosystems. Considering this information, our Urban Barcoding project uses lichens as a bioindicator of environmental pollution, climate change, and ecological continuity.

A lichen is a composite organism composed of fungi and algae (or another bacteria). These two organisms form a symbiotic relationship: since fungi are unable to photosynthesize, they seek an outside food source that can conduct photosynthesis. Through lichen's association with algae or other bacteria, it provides itself with a constant access to a source of nourishment.

Lichens are sensitive to atmospheric pollution because their nutrients come from water and atmospheric deposition. Nitrogen deposition, among other forms of pollution, can contribute to the nutrients. If there is too much nitrogen, chlorophyll will not be able to produce sugars, making it impossible for plants to thrive. Certain lichen species, like fruticose and foliose, are more sensitive to air pollution while others, like crustose lichen, are more tolerant. An increase in nitrogen-tolerant lichen coupled with a decrease in nitrogen sensitive species could indicate increased atmospheric nitrogen deposition.

Through DNA barcoding, the impact of the environment on lichen species was tested. Inconsistencies among the samples' DNA as compared to the database will indicate an external influence that affects the DNA of the lichens.

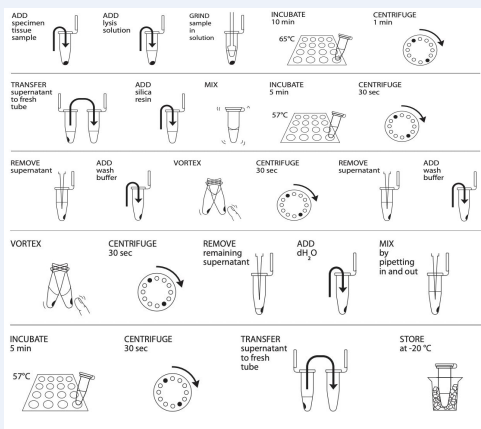
Methods

Our group visited Central Park and Black Rock Forest to observe different species of lichens. 15 samples of lichens were collected from rocks, trees, and soil, and placed individually into paper bags. Paper bags are used so as to not suffocate the organisms. On the paper bag, the sample number and location will be recorded. The samples will then be brought back to school to be tested.

In the school's lab, DNA was extracted from the lichen samples through the Rapid DNA Isolation process on the Urban Barcoding Website. To begin this process, a sample of lichen that measured to be $\frac{1}{8}$ - $\frac{1}{4}$ inch was placed into a tube. Lysis solution was added to the tube. A plastic pestle was then used to break up the tissue in the tube until the sample was broken up into fine, liquefied particles. This step is necessary because it breaks the cell wall, which will provide easier access to the DNA in the nucleus. A paper disk was added to the solution and binded to the DNA so that the DNA is separated from contaminants. A wash buffer was added as the disk soaked so that contaminants that inhibit this process are eliminated. The disc was then removed and transferred to the tube with the wash buffer. The sample was then removed from the buffer and left to dry. The disc was transferred to a tube with TE to soak overnight at 4°C. This allows DNA to be extracted.

Throughout the experiment, it was essential that all equipment was sterilized and cross-contamination was avoided so that no human error could skew the results.

Silica DNA Isolation Process



Results

Despite efforts to meticulously follow the experimental design, this experiment only bore one result. Consequently, the Urban Barcoding Project did not approve our samples being sent for sequencing. Despite this misfortune, the experience working with lichen taught us a variety of valuable skills that will help us in the scientific future.

Discussion

Although the steps of the protocol were followed carefully, the lack of results indicates much error. Some possible sources of error are as such: Firstly, when the lichen samples were collected from Central Park, experimenters placed the samples in plastic bags rather than paper bags. Although the lichens were only in the bags for a few hours, it is possible that this environment suffocated the lichens, preventing them from effectively being sequenced. Additionally, because the Independent Science Research program only meets twice a week, the experimental process was spread over several days. These breaks within the DNA barcoding process might have prevented our results from showing up. Lastly, while using the micropipette, sometimes the amount measured by the pipette was the incorrect amount. This may have affected the end results.

While the experiment bore no results, it is likely that the following species of lichens would have been found: *Lepraria neglecta*, *Punctelia rudecta*, *Phuscia stellaris* in Black Rock Forest, and *Rubropulchra* and *Phaeophyscia ciliata* in Central Park. This data is supported because the other groups of students participating in the Urban Barcoding Project have identified these species. In future, this process should be conducted again so that success can be achieved.

Acknowledgements

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