Identifying Invasive Species in New York City Fresh Waterways Using eDNA Metabarcoding

Abstract

The purpose of this experiment was to determine how the type of flour a respective sourdough was fed, whether gluten-containing or gluten-free (GF), would influence the microbial composition of the sourdough, and whether the respective sourdough samples would be associated with various health benefits. The sequences were analyzed in DNA Subway using the metagenomic line. The oat GF, Italian Ischia GF, buckwheat gluten, and buckwheat GF all contained the bacterial family of Leuconostocaceae. The San Francisco gluten, Italian Ischia gluten, Swedish GF, and Swedish gluten samples were all dominated by the bacterial family Lactobacillaceae, while the oat gluten sample contained the bacterial family Planococcaceae.

Introduction

Environmental DNA

- Metabarcoding using environmental DNA is utilized to discover and identify a variety of species present in an environment. [2]
- Invasive species can threaten the safety and existence of existing species in wildlife by outcompeting other organisms for resources, and can eventually lead to long lasting environmental damage.

Invasive Species

- Invasive species present in New York City's fresh waterways include Eurasian watermilfoil, water chestnut, curly-leaf pondweed, hydrilla, fanwort, Brazilian elodea, and starry stonewort. [3]
- Invasive species present in the Golden Pond lakes, the site of the project's DNA collection, include SnakeHead Fish, Red Ear Slider Turtle, English Ivy, and Phragmites
- The aim of this project was to identify invasive species present in NYC's fresh waterways through eDNA metabarcoding
- Sequencing, DNA analysis and BLAST sequence comparison were utilized to identify various invasive species within the environment

Hypothesis

• The hypothesis of the experiment was that the variety of invasive species present in New York City's waterways has a detrimental impact on the native species of the pond and its surrounding ecosystem

Methodology

Sample Collection

- Approximately one liter of freshwater was collected from Golden Pond in Queens, NY 11361. The samples were taken at least 50 feet apart from each other to ensure variety among our samples.
- The process of collecting the samples included the use of filters, syringes, sterile bottles, and a sterile cooler.

DNA Extraction

- The filters were processed with a Norgen 64100 microbiome DNA extraction kit and amplified with a cocktail of rbcL and matK primers.
- The eDNA was sequenced with DNA sequencing to search for possible invasive species. Since the exact species present are unknown, this included a wide search for plants.

DNA Analysis

- Nanopore DNA sequencing was performed to analyze DNA with four steps. • Fastp software to filter out low-quality data Nanopore Adapters
- Barcode DNA was removed using Porechop ABI.
- Similar sequences (likely from the same species) were sorted using Amplicon sorter.
- BLAST was used to search for matches in our DNA to known samples. Nanopore Sequencing [9]
- This method of sequencing provides real time analysis, effectively allowing for a significantly faster analysis of results.
- This technology has the ability to both read native types of DNA and RNA for analysis.
- A crucial advantage of nanopore sequencing is its ability to sequence a fragment of any length.

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Results



Image 1. Locations of Sample Collecting. This image is a map of Golden Pond. The blue flags depict the location of where each sample was collected. Sample 1: (40.7704954, -73.7641267) Sample 2: (40.7705547, -73.7650713) Sample 3: (40.7712154, -73.7652577)



Image 2. *Ampelopsis glandulosa**, an invasive species native to temperate areas of Asia. This plant is more commonly known as the porcelain berry.



Image 3. Golden Pond, the site of the sample collection for this experiment. Golden Pond is located in Queens, New York.

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Total Run Time	24.25 hrs
Temperature	30
Read Count	1.97 M
Estimated Bases	757.51 Mb
Bases Passed	404.94 Mb
Bases Failed	207.18 Mb

Table 1. Nanopore Sequencing Results. This table depicts the results of the nanopore sequencing and the time and temperature required to obtain those results.

Introduced into North. America	No Record of Formal Introduction into North America
Ceratophyllum demersum	Parachlorella kessleri
Chlamydomonas applanata	Ankistrodesmus stipitatus
Nymphaea mexicana	Mesostigma viride
Choricystis parasitica	Chlorella variabilis
Nephrocytium limneticum	Malmea dimera
Chloroidium saccharophilum	Pseudowintera colorata
Raphidonema nivale	Raphidonema planctonicum
Populus fremontii	Ampelopsis glandulosa*
Pontederia cordata	
Chamaesiphon minutus	
Volvocales	
Orobanche austrohispanica	
Lobomonas francei	
Oedocladium carolinianum	
Carteria	
Micractinium pusillum	
Ankistrodesmus stipitatus	

 Table 2. Species From BLAST Results Native and Non Native
to N. America. All algae species were run through AlgaeBase in order to determine whether they've been formally introduced into N. America [11].

*This species, Ampelopsis glandulosa, is very invasive and is illegal to be sold in NY [10].

Discussion

Error, Limitations, and Future Studies • The timing of the sample collection (late fall) could have been problematic to our data, as many plants could have already been dead when the samples were collected. • In the future, such an experiment looking at invasive species, especially plants, should be performed in warmer seasons to ensure a proper collection of data.

• Nanopore Sequencing has a large margin of error, which can be up to 15%. It is thus possible that the three samples did not provide accurate data of the invasive species present.

• The use of the internet as a resource for classifying the species found as an invasive species was a major limitation as there is no clear definition or guideline for invasive species.

• The primers used, rbcl and matK, are only applicable to plant DNA. However, invasive species can include plants, animals, fungi, and bacteria. • Future research should explore the presence of animal, fungi, and bacteria invasive species to fully understand what invasive species are present in NYC waterways.

Support of Hypothesis • The hypothesis of this experiment was proven to be correct as the results demonstrated a presence of invasive species in the NYC waterways.

Authority, quence.

• English Ivy, an invasive species commonly found in NYC waterways, which were not present at all in any of the three samples. [3]

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