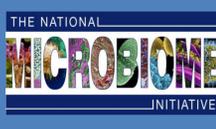


Microbiome Analysis of Water with Different Salinities on the South Shore of Long Island



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Abstract

DNA barcoding is used to identify the types of microorganisms in a specific ecosystem, also called a microbiome. New DNA sequencing technology allows us to determine the identity and diversity of microbial life to better understand their role in ecosystems. The purpose of this study was to develop a protocol to identify the types of microorganisms living in aquatic ecosystems of varying salinity. Water was collected at Gardiner Park in fresh, brackish, and salt water bodies, then filtered through a Peristaltic Pump and Sterivex filter membranes to collect microorganisms. Microbial DNA was extracted using a MoBio PowerSoil DNA Isolation Kit, and the 16S rRNA gene was amplified by PCR. Successful amplifications verified through gel electrophoresis were sent for Illumina sequencing. Jupyter Notebook and SEPA 08 QIIME version were used to analyze the sequence results and the data was organized into charts to identify the microorganisms by abundance. Bar plots were generated that organized the data to the genus taxa. Species were unable to be determined; therefore pathogenicity is still in question. Results showed that Flavobacteriaceae, Rhodobacteraceae, and Stramenopiles were found in each salinity, supporting the hypothesis that similar bacteria would be found among all three salinities. Methylophilaceae was found in fresh and saltwater environments, but not brackish. Some species of Methylophilaceae are harmful to humans who swim and fish in these waters; therefore future research should include improving microbiome barcoding techniques in order to identify the microbes to the species level in order to conclude if they are pathogenic.

Introduction & Hypothesis

- A microbiome is the habitat in which various microorganisms live. DNA barcoding can be used to identify the different types of microorganisms that are present in a specific location or ecosystem. This method is used when you are able to use a fragment of a genome for the classification of an organism (Boldgiv, 2004).
- By producing a protocol for barcoding the different microorganisms in freshwater, brackish water and saltwater, we may be able to conclude whether or not similar bacteria live in all three of these ecosystems. Mapping out the microbiomes of each of these salinities, we can analyze which microorganisms are present and in what abundance.
- It was hypothesized that we would find similar bacteria in the freshwater and brackish water, and the brackish water and saltwater due to their natural mixing.
- Greater species diversity ensures natural sustainability in an ecosystem, which is the underlying importance of this study.

Materials & Methods

Sample Collection:

A transect line was set up at Gardiner Park. Water was collected in three sterile one gallon containers at each salinity: fresh water, brackish, and bay water.

Sample Documentation:

Photographs of each ecosystem were taken and all metadata was recorded in log books, including what other organisms were in the area, water salinity and pH.

DNA Collection:

A peristaltic pump assembly and sterivex filter units from EMD Millipore were used to filter the water. After each sample was filtered, the filter membrane was placed in a Petri dish and frozen until ready for DNA extraction.

DNA Barcoding:

DNA Extraction - MoBio PowerSoil DNA Isolation Kit
 PCR – Isolate 16S rRNA gene
 Gel Electrophoresis
 Illumina Sequencing



Figure 1: Transect line at Gardiner Park. Location 1 is bay water, 2 is brackish water, and 3 is the fresh water. Photo courtesy of Google Maps.



Figure 2: Collecting water samples at a brackish salinity in Gardiner Park. Photo taken by research teacher.



Figure 3: Setup of the peristaltic pump assembly and the Erlenmeyer flask used to filter the water samples. Photo taken by researchers.



Figure 4: The results of gel electrophoresis for three of the samples. Bands of approximately 1500 bp are present, indicating the 16S rRNA gene is present in those samples.

Data Analysis

- Jupyter Notebook was used to analyze the sequence results, specifically the SEPA 08 QIIME version. We went through a series of lessons that helped us view our samples.
- It helped us create a mapping file, which contained essential information about the samples and associated metadata, then once all the samples were in the mapping file we were able to extract the primer sequence. By removing the primers and amplifying the barcodes, we were able to determine the necessary reads for classification.
- Then, the split libraries assign code reads the samples based on their nucleotide barcode, performing quality filtering and removing any low quality or ambiguous reads.
- Finally, the last lesson consisted of summarizing communities by taxonomic composition. This generated the bar plots (Figure 6).
- The sequences were received from the laboratory and the microorganisms that were present in the samples were identified and analyzed.

Table 1: Metadata collected along with samples at Gardiner Park. Freshwater (F), Brackish water (B), and Salt water (S)

| Sample ID | Salinity (ppt) | Water Temp. (C) | pH | Notes |
|-----------|----------------|-----------------|-----|--|
| F | 0 | 10.9 | 8.1 | Ducks swimming, Swan, Coy fish |
| B | 14.8 | 10.8 | 7.2 | Lots of detritus, decaying leaves |
| S | 29.3 | 6.6 | 8.3 | Small birds on shore and geese flying over |

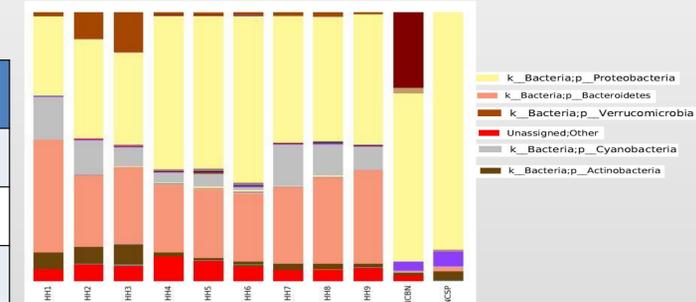


Figure 6: Bar plot of the bacterial phyla found in samples. Fresh Water (HH1-HH3) Salt Water (HH7-HH9) Brackish Water (HH4-HH6) Negative Controls (NCBN-NCSP)

Table 2: Average % of bacteria found in each type of water sample.

| Bacterial Classification | Fresh Water | Brackish | Salt Water | Bacterial Information |
|----------------------------------|-------------|----------|------------|---|
| k_Bacteria g_Flavobacterium | 8.47% | 16.22% | 10.41% | Flavobacterium is commonly found in a wide variety of marine, freshwater and soil habitats and some could be pathogenic to fish, birds, and humans (Bernardet & Bowman, 1970; McBride, 1970). |
| k_Bacteria g_Rhodobacter | 3.24% | 7.67% | 5.47% | Rhodobacteraceae is common in fresh and saltwater environments ("Rhodobacter", n.d.). |
| k_Bacteria f_Stramenopiles | 1.03% | 1.04% | 9.88% | Stramenopiles are photosynthetic algae that serve as a food source for many marine organisms (Microscope, n.d.). |
| k_Bacteria g_Fluviicola | 6.53% | 1.46% | 0% | Fluviicola is common to marine environments and play a role in waste decomposition (Woyke, 2011). |
| k_Bacteria f_Comamonadaceae | 6.78% | 2.51% | 0% | Comamonadaceae is mostly found in soil and marine environments, but some have been found in Antarctic habitats and even hot springs (Willems, 1970). |
| k_Bacteria f_Methylophilaceae | 1.64% | 0% | 2.31% | Methylophilaceae can be opportunistic human pathogens (Kelly, McDonald & Wood, 1970). They have been known to use methanol, which is toxic, as a source of energy. The large amount of animal waste in these areas creates methanol, which the organism is able to use (Ajayi & Adefila, 2012). |

Conclusions

- The hypothesis was supported because there were similar bacteria found in the brackish and fresh, and the brackish and saltwater.
- One interesting aspect was that the *Methylophilaceae* bacteria was only found in the fresh and saltwater environments, but not in the brackish. It would make sense for this microorganism to inhabit the brackish due to its presence in both the fresh and salt salinities, of which the brackish is a combination of both waters.
- Similar microorganisms are able to survive in a variety of habitats. Different microorganisms have varied adaptations that allow them to live in multiple salinities and survive. Without this adaptation, multiple species of microorganisms could face extinction from the same source.
- The organisms that live in these environments, such as fish, can take in microorganisms through their gills. If these microorganisms are harmful, they can negatively effect the entire ecosystem.

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