

Bacterial Diversity in East River as a Possible Window into Pollutant Sources

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ABSTRACT

East River is a brackish water tidal estuary connecting the Upper New York Bay to the Long Island Sound. In this study, we used metagenomics to analyze the microbiome of a water sample from East River collected on the Long Island City coast. The purpose of our study was to classify bacterial species found in this water according to their typical habitat. DNA was extracted from organisms in the water sample and sent for metagenomic analysis. We analyzed the profile bacterial classes found in the samples and discovered among them high amounts of bacterial species typically present in freshwater, marine, and brackish water environments. The most common freshwater bacterial classes were Actinobacteria and Betaproteobacteria. From marine environments, there were high numbers of Flavobacteria, *Alphaproteobacteria* and *Gammaproteobacteria*. Out of these, *Actinobacteria*, *Gammaproteobacteria* and *Alphaproteobacteria* are the most common classes found in uncontaminated brackish water. We also used the bacterial analysis information to identify the presence of bacterial classes atypical from healthy brackish water bodies. These include potentially pathogenic bacteria that are usually present in sewage, animal gut, and toxic waste. Some examples of such bacteria are pathogens like *Bacteroidia*, *Chlamydia*, *Clostridia*, and *Fusobacteriia*, gut bacteria like *Lentisphaeria*, *Candidatus Saccharibacteria*, and *Erysipelotrichia*, and sewage bacteria like *Elusimicrobia*. The results from this study could assist future research about pollutant bacterial species in East River and aid in determining the origin of the contaminating bacteria as well as strategies for its remediation

Keywords: East River, brackish water, water contamination, metagenomics, bacterial diversity.

INTRODUCTION

A factor that plays an important role in determining bacterial diversity in aquatic ecosystems is salinity. Variations in salt concentration can affect bacterial energy consumption and metabolic pathways, thus limiting the presence of bacterial groups in water bodies according to salinity levels (Mehrshad et al., 2016). Freshwater bodies typically have a salt content lower than 1.0 g/L. Freshwater bacterial populations are dominated by Gram negative bacteria, although certain Gram-positive bacteria like *Actinobacteria* are also abundant (Okafor, 2011). Saltwater bodies like oceans and seas are characterized for containing salt concentration levels of more than 35.0g/L (Okafor, 2011). Bacterial populations in saltwater are comprised of “salt-loving” or halophilic bacteria that typically abound near the surface (Hoyle, 2014). This group is dominated by photosynthetic bacteria like *Cyanobacteria*, Gram negative bacteria like *Alphaproteobacteria* and Gram positive green non-sulfur bacteria (Okafor, 2011). Brackish water bodies are those with salinity levels higher than freshwater but usually lower than saltwater. Their salinity can vary each day depending on factors like weather and the tides (Levinton, 2017). This variation in salt concentrations occurs in marine-freshwater transition zones, like estuaries and coastal areas (Okafor, 2011). In these zones, bacterial communities from saltwater and freshwater mix, and their relative abundance can fluctuate due to variations in the tide (Mehrshad et al., 2016).

East River is a brackish tidal estuary connecting the Upper New York Bay to the Long Island Sound. It is fed by saltwater coming from the Atlantic Ocean through the Harlem River, and freshwater coming from the Bronx River and from the Thames, Housatonic and Connecticut rivers via the Long Island Sound tidal estuary (Riverkeeper Inc., 2019). East River has been historically burdened by different pollutants like garbage, oil, heavy metals, bacteria and toxic waste coming from industrial waste, storm water runoff, separate and combined sewer systems which has made this tidal strait one of the most polluted estuarine systems in the world (Masseria & Hesse, 2012).

The main focus of our investigation was to analyze the microbiome of a sample corresponding to the surface of East River, particularly its bacterial

diversity. The term bacterial diversity refers to the different species clusters of bacteria in an ecosystem. Because the diversity of bacterial communities is shaped and maintained by the natural environment, bacteria can be an indicator of the characteristics of an ecosystem (Gibbons & Gilbert, 2015). Recent advances in molecular technology allow us to study bacterial diversity through the sequencing of DNA from bacterial species. Over the course of three months, we established some of the physical and chemical characteristics of water samples from East River. We purified DNA to conduct a metagenomic analysis using a bacterial gene, 16S rRNA, for amplification in PCR to ensure the analysis of exclusively bacterial DNA (Bruijn, 2011). With this information in hand, we analyzed the bacterial classes and found not only bacteria from marine and freshwater environments but also bacteria that are atypical of brackish water. This type of analysis is useful to give us an indication of the possible sources of contaminants according to the kinds and amounts of these atypical bacteria.

MATERIALS AND METHODS

Water samples used in this study were obtained from the surface of East River in Long Island City on the following dates: The first sample was taken on October 2, 2019, at 7:45 a.m. The salinity of this sample was determined by measuring the mass of remaining salt after evaporating 10.0 mL of sample in a watch glass and extrapolating this value to one liter of sample. Temperature and pH were measured with a thermometer and pH paper, respectively. The second sample was taken on October 15, 2019, at 1:25 p.m., and the third sample was taken on October 23, 2019, at 9:06 a.m. Both O₂ and CO₂ concentrations were measured using LaMotte titration kits. DNA was isolated from the first water sample using the MoBio WaterPower Kit. The purified DNA was used to conduct a metagenomic analysis using 16s rRNA gene sequencing to identify bacterial species that were present in the collected water sample. Bacterial populations were sorted and classified using the Taxonomy Common Tree search tool from the National Center for Biotechnology Information (NCBI). The typical habitat of each bacteria genus was identified using David H. Bergey's *Manual of Systematics of Archaea and Bacteria*, *Bacterial Systematics* by Niall A. Logan, and *The Prokaryotes: A Handbook on the Biology of Bacteria* by Albert Balows.

RESULTS

First, we found the source characteristics of the water sample. Source characteristics show the general relevant properties of the water sample source, East River, in relation to this study. They are as follows:

The pH was 8.0, and the temperature was 23.0 °C. The salinity was measured to be 26,200 mg/L, the O₂ concentration was 5.4 ppm, and the CO₂ concentration was 21.25 mg/L.

Physical and chemical characteristics of the East River surface area near Long Island City

pH	Temperature	Salinity	O ₂ concentration*	CO ₂ concentration**
8.0	23.0 °C	26,200 mg/L	5.4 ppm	21.25 mg/L

Table 1. The table above displays the physical and chemical characteristics of the sample collected from the surface of East River.

*Measured on water sampled on Oct 15, 2019, at 1:25 p.m.

**Measured on water sampled on Oct 23, 2019 at 9:06 a.m.

The next step was to examine the bacterial diversity of the water sample. First, results from the metagenomic analysis were organized by taxonomic class, then compared to populations typically found in a brackish water body.

Bacterial diversity organized by class based on 16S rRNA gene reads from the East River surface area near Long Island City

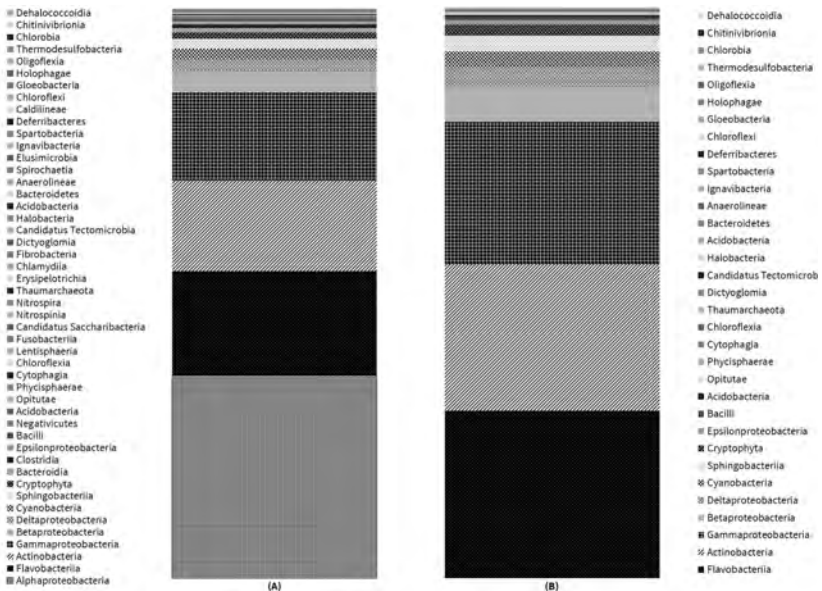


Figure 1. (A) and (B). Figure 1(A) shows all bacterial classes based on 16S rRNA gene reads identified from the sample obtained on the surface of East River. Figure 1(B) shows only the bacterial classes typical of brackish water out of all bacterial diversity found on the East River surface

Next, bacterial classes were broken down into each bacterial genus, and a more profound analysis was conducted in order to identify the genera that are not commonly found in a typical and unpolluted brackish water body. The results of this analysis are shown on the next figure:

Bacterial genera atypical of brackish water found on East River Surface near Long Island City

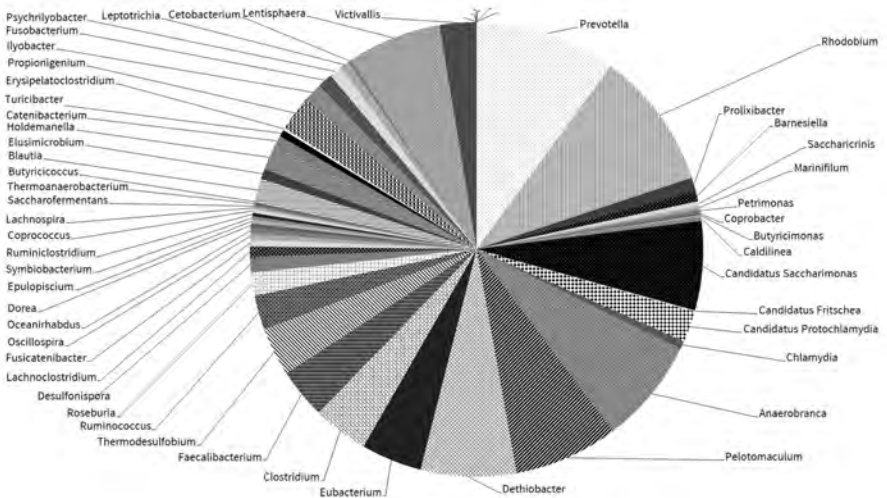


Figure 2. Bacterial genera atypical from brackish water identified from the surface of the East River near Long Island City. This figure breaks down all bacterial classes atypical to brackish water shown in Figure 1(A) into each individual genus.

The results displayed in Figure 2 give us a clearer idea of which are the atypical bacteria that need to be examined further in order to find contaminants. That information can be found in the following section.

DISCUSSION AND CONCLUSIONS

Regarding the characteristic of the water sample, levels of dissolved CO₂ and O₂ were within the standard considered appropriate for survival of most aquatic species. The pH of the water sample was also within what is expected from brackish water, which is slightly alkaline. Salinity measured as total dissolved solids was within normal levels for brackish water. The next

step was to analyze the bacterial diversity of the sample.

Our results were consistent, regarding which bacterial classes were the most abundant in the sample. We found that for all three types of water bodies (freshwater, saltwater and brackish water), the most abundant bacterial classes were *Alphaproteobacteria*, *Flavobacteriia*, *Actinobacteria*, *Gammaproteobacteria* and *Betaproteobacteria*, as shown in Figure 1(A). Literature research indicates that from these classes, *Actinobacteria* and *Betaproteobacteria* are representative classes of freshwater, *Alphaproteobacteria*, *Gammaproteobacteria* and *Flavobacteriia* are typical from saltwater, and finally *Alphaproteobacteria* and *Actinobacteria* are abundant in brackish water. This is close to our findings here, with the difference that *Flavobacteriia* was the second most abundant bacterial class, followed closely by *Actinobacteria*, as depicted in Figure 1(B).

Next, we examined the bacterial classes that are not commonly found in healthy brackish water bodies. These include the following classes: *Bacteroidia*, *Chlamydia*, *Clostridia*, *Fusobacteriia*, *Lentisphaeria*, *Candidatus Saccharibacteria*, *Erysipelotrichia*, and *Elusimicrobia*. From these classes, we divided again by genus, as shown in Figure 2, and examined the normal habitats of these genera. Within *Bacteroidia*, we found *Prevotella*, an opportunistic pathogen normally found in human intestinal flora. In the class *Candidatus Saccharibacteria*, we found *Candidatus Saccharimonas* which can be found in human oral cavities and activated sludge. Within the class of *Chlamydia*, we found three different genera that are common parasitic bacteria in homeotherms. Within the *Clostridia* class, we found the genus *Clostridium*, which contains some common human pathogens, and *Faecalibacterium*, abundant in human gut biota. In *Elusimicrobia*, we found the genus *Elusimicrobium*, which is commonly found in sewage sludge, contaminated soils, and toxic wastes. The class *Erysipelotrichia* contains such genera as *Holdemanella*, found in human gut and wastewater, *Catenibacterium*, found in human feces, and *Turicibacter*, animal gut bacteria. From *Fusobacteriia*, genera containing bacteria from human oral and gut microbiota like *Leptotrichi*, *Cetobacterium* and *Fusobacterium* were found, from which the latter is also considered an opportunistic pathogen.

From *Lentisphaeria* we found the only two genera in that group which are *Lentisphaera*, common marine bacteria, and *Victivallis*, bacteria from the human digestive tract.

The discovery of contaminant bacteria characteristic of sewage sludge, toxic waste, human gut, animal gut, and feces is concerning given that the sample was taken from the surface of the East River in a heavily populated area such as Long Island City. This water can be easily and legally accessed by the general public, leaving it exposed to opportunistic pathogens and susceptible to disease. New York is one of the nation's most populated cities, and its surrounding water bodies continue to be polluted. At a time when many are oblivious to water pollution and the negative effects, we humans have on our environment, these findings should not be ignored. This research is a starting point for future studies into the contamination of water bodies around New York City and their future remediation. Further sampling and metagenomic analysis of different areas from the East River over longer periods of time would allow for comparisons of its bacterial diversity and give more insight into the sources of pollution of the East River.

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The evidence of bacterial contamination suggests the possibility of chemical contamination as well, and a chemical analysis of the East River could be conducted to find sources of chemical contamination. These

findings are of interest to the health sciences, because pathogenic bacteria pose a risk of infectious diseases for the nearby population. By doing this work, we gained a greater understanding of our surroundings, an awareness of a global issue that is a biological imperative for all of us, and an appreciation for the complexity of the East River's microbial system.

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