

Abstract

East River is a brackish water tidal estuary containing a mixture of saltwater from the Atlantic Ocean and freshwater from three rivers (Thames, Housatonic, and Connecticut) arriving to East River via the Long Island Sound tidal estuary. In our 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 the 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 *Alphaproteobacteria* and *Gammaproteobacteria*. From these, *Gammaproteobacteria*, *Actinobacteria*, 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 potentially 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.

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 (Mehrhshad 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 3.5 g/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). Water bodies with salinity levels higher than freshwater but usually lower than saltwater are denominated brackish water bodies. 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 (Mehrhshad et al., 2016).

East River is a brackish water 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, stormwater 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).

Over the course of three months, we established some of the physical and chemical characteristics of water samples from East River. We also purified DNA in order to conduct a metagenomic analysis using a bacterial gene, 16SrRNA, for amplification in PCR to ensure the analysis of exclusively bacterial DNA (Brujin, 2011). With this information in hand, we conducted an analysis of the bacterial classes and found, not only bacteria from marine and freshwater environments, but also bacteria which 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

The water sample used in this study was obtained from the surface the East River in Long Island City on October 2, 2019 at 7:45am.

The salinity of the water sample was determined by extrapolating the value from a standard curve of salt concentration. The pH and temperature were measured with pH paper and a thermometer, respectively. Both CO₂ and O₂ concentrations were found using *LaMotte* titration kits. DNA was isolated from the collected 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 water sample collected. Sorting and classification of bacteria was done with 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

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

pH	Temperature	Salinity	CO ₂ concentration*	O ₂ concentration**
8.0	23.0 °C	61,800 mg/L	21.25 mg/L	5.4 ppm

Table 1. The above are the physical and chemical characteristics of the water sample collected from the surface of East River. *Measured on water sampled on October 23, 2019 at 9:06am. ** Measured on water sampled on October 15, 2019 at 1:25pm.

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

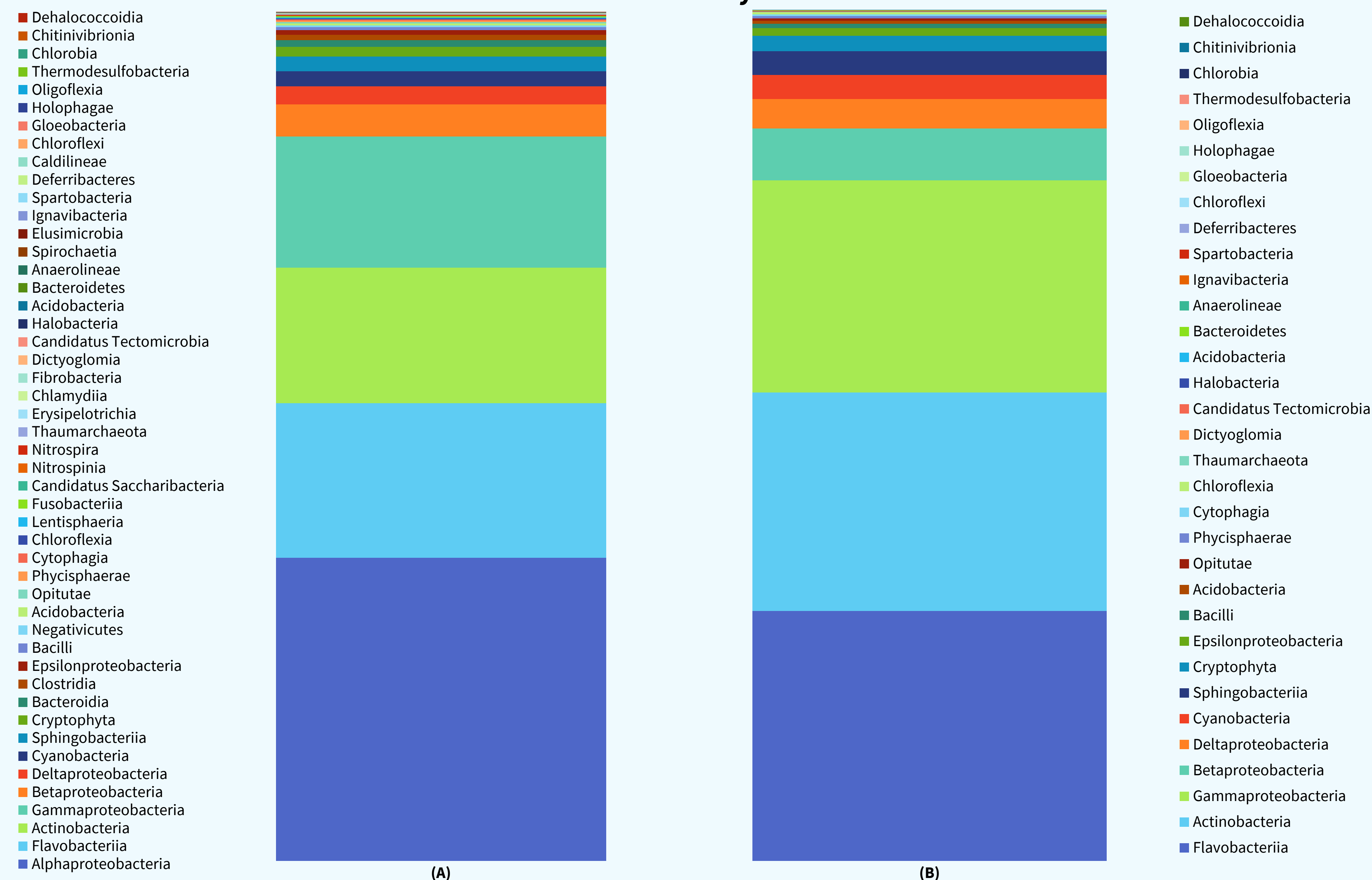


Figure 1. (A) and (B). Figure 1(A) shows all bacterial classes based on 16S rRNA gene reads identified from the East River surface. Figure 1(B) shows only the bacterial classes typical of brackish water out of all bacterial diversity found on the East River surface.

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

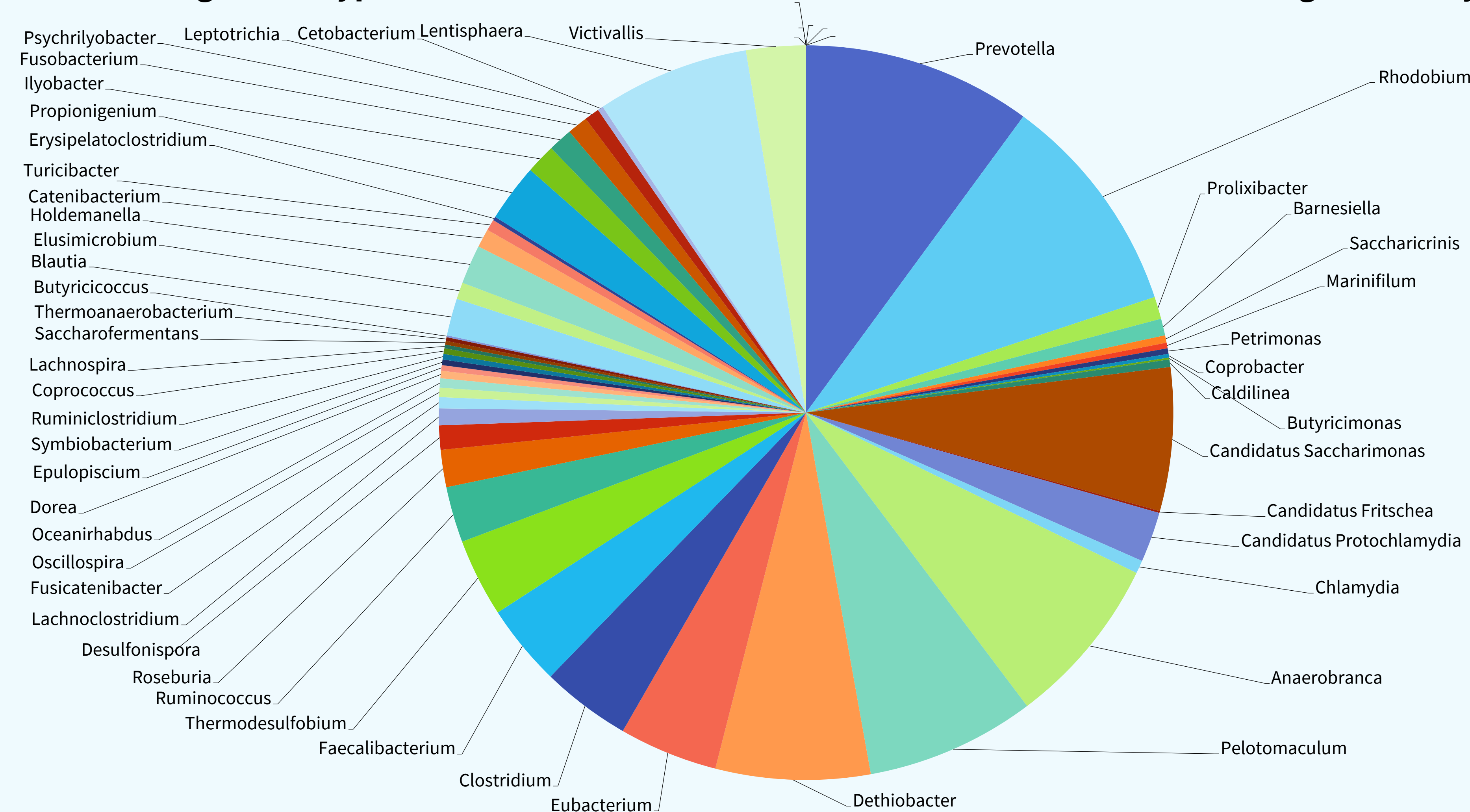


Figure 2. Bacterial genera atypical from brackish water identified from the surface of East River near Long Island City. This figure breaks down all bacterial classes atypical to brackish water shown in Figure 1(A) into each individual genus.

Discussion and Conclusions

From our results we found that for all three types of water bodies (freshwater, saltwater and brackish water), the most abundant bacteria groups common to all three were *Alphaproteobacteria*, *Flavobacteria*, *Actinobacteria* and *Gammaproteobacteria* and *Betaproteobacteria*, as shown in Figure 1(A).

Literature research indicates that from these groups *Actinobacteria* and *Betaproteobacteria* are representative from freshwater, classes *Alphaproteobacteria*, *Gammaproteobacteria* and *Flavobacteria* are typical from saltwater, finally *Alphaproteobacteria* and *Actinobacteria* are abundant in brackish water. This is very close to our findings here, with the difference that *Flavobacteriia* was the second most abundant bacteria group, followed closely by *Actinobacteria*, as depicted in Figure 1(B).

Next, we examined the bacteria groups that are not commonly found in healthy brackish water bodies. These include the following groups: *Bacteroidia*, *Chlamydia*, *Clostridia*, *Fusobacteriia*, *Lentisphaeria*, *Candidatus saccharibacteria*, *Erysipelotrichia*, and *Elusimicrobia*. From these groups, 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*, an 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*, a normal marine bacteria, and *Victivallis*, a human digestive tract bacteria.

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.

Future work

- ⚡ 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.
- ⚡ 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.

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