

Abstract

Urban public spaces like subway stations and playgrounds in New York City experience heavy daily use, creating unique environments rich in microbial life. Despite their importance, these outdoor microbial communities have been less studied than those in medical or indoor settings. This research collected DNA samples from subway pillars and playground soils across all five boroughs to investigate bacterial diversity using sequencing techniques. The analysis revealed distinct differences in microbial populations depending on location and surface characteristics, highlighting important considerations for public health. Some fascinating finds were the presence of shizuokensis and faecalis bacteria (primarily in Rockefeller Park and Jay Street-Metrotech station, respectively). This study emphasizes the need for governmental investment in science-based sanitation measures, including regular microbial monitoring and enhanced cleaning in frequently touched areas to better protect public health and improve urban living conditions.

Introduction

Public spaces like playgrounds and subway stations are major hubs of human activity and serve as complex microbial ecosystems. Understanding the types and diversity of bacteria in these areas is crucial for assessing public health risks, improving sanitation strategies, and gaining insight into urban microbial ecosystems. This study focuses on New York City, using its dense population and extensive public infrastructure as a setting to explore bacterial biodiversity across different boroughs and public areas within those boroughs. Environmental DNA (eDNA) methodologies have revolutionized our ability to assess the composition and diversity of microbial communities in various habitats, providing valuable insights into the microorganisms present at collection sites. By collecting surface samples from playgrounds and the pillars of subway stations and analyzing bacterial DNA through Next-Generation Sequencing (NGS), the research aims to compare microbial communities in differing environments based on factors like environmental exposure and surface material.

The Use of eDNA in the Identification of Microbes Within New York City Public Spaces

Dafina Burlakova¹, Syeda Hoque², Xiao Chen³ ¹Stuyvesant High School, ²Brooklyn Technical High School, ³John Jay College

Materials & Methods

Samples were collected from subway stations and playgrounds across New York City to study microbial diversity. Subway samples were obtained using plastic pipettes from less-crowded areas at the bases of subway pillars. Two samples were collected from the Jay Street–MetroTech station in Brooklyn. Playground samples consisted of 0.25 grams of soil collected into 2 mL tubes from Teardrop and Rockefeller Parks (Manhattan), Fort Greene Park (Brooklyn), and Woodhaven Park (Queens).



DNA extraction was performed using the DNeasy PowerSoil Pro Kit. Soil samples underwent chemical and mechanical homogenization, bead beating, and purification using silica spin filters. DNA concentrations were measured using a Qubit fluorometer. Next-generation sequencing (NGS) was performed at GeneWiz, with Illumina sequencing protocol. The sequencing data, FASTQ were processed and analyzed for microbial abundance using the QIIME pipeline. Figure was generated with the use of R Studios.



The heatmap, generated using the QIIME pipeline, illustrates the differences in bacterial abundance across five NYC sites: Jay Street-MetroTech subway station (Brooklyn), Teardrop and Rockefeller Parks (Manhattan), Fort Greene Park (Brooklyn), and Woodhaven Park (Queens). Teardrop Park exhibited high levels of roseus, variabile, and psychrophilus, while Woodhaven showed strong presence of cold-tolerant species like *psychrophilus*, *faecalis*, and *glaciei*, likely due to cooler, shaded soil conditions. In contrast, Jay Street displayed lower bacterial diversity, with moderate levels of species like *flexus*, *aquatilis*, and *plantarum*. Notably, *roseus* and *faecalis* were found at all locations, suggesting they are widespread in NYC's urban environments and densely populated transportation centers. Unclassified sequences was the most abundant. Unclassified may conclude the lack of database on undiscovered organisms with the 16S primers.Overall, playgrounds showed greater microbial diversity than the subway site.

This study successfully used eDNA to map out microbe diversity and analyzed microbial DNA from surfaces across boroughs to investigate how microbial communities vary by location and surface type. This method can be expanded to indoor collection sites, as well. The findings highlight significant differences in bacterial diversity influenced by environmental factors, which has important implications for public health.

Figure 1 illustrates microbial diversity across collection sites, showing that bacteria correlate with their natural environments. For example, viridiflava, a plant-infecting bacterium, was found in outdoor soil near playgrounds, while psychrophilus and *pulmonis*, bacteria associated with metal reduction and rodent infection, were most abundant in the indoor setting of Jay Street–Metrotech station. Despite being geographically close to Fort Greene, the subway station harbored more environmentally distinct bacteria, suggesting that environmental conditions, rather than location, play a greater role in shaping microbial communities.

Fascinating finds were the presence of *shizuokensis* and *faecalis* bacteria (primarily in Rockefeller Park and Jay Street-Metrotech station, respectively). Alicyclobacillus shizuokensis is a bacterium that was initially found in the Japanese city of Shizuoka, in the Shizuoka Prefecture. Its spread to a public park in NYC indicates the patterns of human travel to and from Japan. *Enterococcus faecalis* is commonly found in the human gastrointestinal tract, and is expelled from the body through fecal matter.

An interesting study to pursue would be to map out the spatial patterns of the most concentrated bacterial species around NYC, giving both a better insight into the climatic differences between parts of the city, and the natural conditions of each area. Conclusions can be drawn by researching the characteristics of the found bacteria, like the preferred environments. Certain bacteria are not thoroughly documented in public databases, highlighting the need for additional studies to advance scientific understanding.

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Acknowledgements

Immense thank you to the Urban Barcode Research Program and Cold Spring Harbor Laboratory for this opportunity and the Pinkerton Foundation for funding us with this wonderfully educational experience, as well as providing us with all of our necessary materials. Thank you so much to our mentor, Xiao Chen, for being with us every step of the journey!



Discussion

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