Air Microbiome of Hong Kong’s Subways

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Hong Kong

Population: 7.2M
Area: 1,104 km²
70% inhabitable because of terrain
Densely-populated

Subway network
Mass Transit Railway (MTR)

Annual ridership: 1.6 billion (2013)
9th busiest system in the world
Indoor-Air Microbiome in an Urban Subway Network: Diversity and Dynamics

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Subway systems are indispensable for urban societies, but microbiological characteristics of subway aerosols are relatively unknown. Previous studies investigating microbial compositions in subways employed methodologies that underestimated the diversity of microbial exposure for commuters, with little focus on factors governing subway air microbiology, which may have public health implications. Here, a culture-independent approach unraveling the bacterial diversity within the urban subway network in Hong Kong is presented. Aerosol samples from multiple subway lines and outdoor locations were collected. Targeting the 16S rRNA gene V4 region, extensive taxonomic diversity was found, with the most common bacterial genera in the subway environment among those associated with skin. Overall, subway lines harbored different phylogenetic communities based on α- and β-diversity comparisons, and closer inspection suggests that each community within a line is dependent on architectural characteristics, nearby outdoor microbiomes, and connectedness with other lines. Microbial diversities and assemblages also varied depending on the day sampled, as well as the time of day, and changes in microbial communities between peak and non-peak commuting hours were attributed largely to increases in skin-associated genera in peak samples. Microbial diversities within the subway were influenced by temperature and relative humidity, while carbon dioxide levels showed a positive correlation with abundances of commuter-associated genera. This Hong Kong data set and communities from previous studies conducted in the United States formed distinct community clusters, indicating that additional work is required to unravel the mechanisms that shape subway microbiomes around the globe.
Aim: Characterize Hong Kong subway air microbial community

Objectives

- Determine microbial diversity of subway network - **integrated sampling**

- **Comparisons of community by variables:**
  - Spatial (MTR vs. outdoor, line vs. line)
  - Architectural properties of lines/stations
  - Temporal (within-day, day-by-day)

- Roles of outdoor locations in subway microbiome

- Relationships between connectedness of subway lines and microbiome

- MTR community vs. other studies
Sampling

7 lines of Mass Transit Railway (MTR) network (10 lines)

4 time slots
- 0745-0945 (AMP) (Peak)
- 1015-1215 (AMNP) (Non-peak)
- 1515-1715 (PMNP) (Non-peak)
- 1745-1945 (PMP) (Peak)

7 outdoor samples
- Admiralty
- Causeway Bay
- TST East
- Kwun Tong
- Po Lam
- Wu Kai Sha
- Science Park

4 replicates per condition (~15 days apart)
Indoor/Under-ground & Outdoor/Above-ground MTR Lines
New lines

5 new lines or extensions will come online within the next few years

Opened Jan 2015
Sampling

4.32 m³
volume air per sample

= RIDE (platform + train sampling)

- Air sample onto nitrose cellulose filters
- Genomic DNA extraction -> Illumina MiSeq based on 16S V4 region
- Taxonomic classification: filtered Greengenes database (genus+species) -> operational taxonomic units (OTUs) based on ≥ 97% sequence identity

Integrated sampling/Passengers’ exposure
Overview

139 samples

Top 4 bacterial phyla
- **Proteobacteria (NYC)**
- **Actinobacteria (NYC)**
- **Firmicutes (NYC)**
- **Deinococcus-Thermus**

Common genera
- **Micrococcus** (99.9% *M. luteus*)
- **Sphingobium**
- **Enhydrobacter**
- **Propionibacterium**
- **Staphylococcus**
- **Corynebacterium**
Overview

Potential opportunistic pathogens

- **Bacillus cereus**
- **Mycobacterium bovis**
- **Micrococcus luteus**
- **Staphylococcus aureus**
- **Streptococcus pneumoniae**
- **Streptococcus sanguinis**
α-diversity:

1) # OTUs
2) Faith’s Phylogenetic Diversity (FPD)
3) Chao1 (total diversity estimator)

Outdoor > MTR samples
PM > AM samples
Day-by-day changes in α-diversity within a line

Exposure of regular commuters
**β-diversity**

**abundance-unweighted and abundance-weighted UniFrac distances**

<table>
<thead>
<tr>
<th>GROUP Comparisons</th>
<th>Global R (Unweighted)</th>
<th>P-value\textsuperscript{a}</th>
<th>Global R (Weighted)</th>
<th>P-value\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTR vs. Outdoor</td>
<td>-0.023</td>
<td>Not sig.</td>
<td>0.046</td>
<td>Not sig.</td>
</tr>
<tr>
<td>Outdoor vs. Outdoor</td>
<td>0.161</td>
<td>0.02</td>
<td>-0.003</td>
<td>Not sig.</td>
</tr>
<tr>
<td>Line vs. Line</td>
<td>0.207</td>
<td>0.001</td>
<td>0.082</td>
<td>0.001</td>
</tr>
<tr>
<td>Underground vs. Above-ground</td>
<td>0.232</td>
<td>0.001</td>
<td>0.064</td>
<td>0.004</td>
</tr>
<tr>
<td>AM vs. PM</td>
<td>0.062</td>
<td>0.001</td>
<td>0.029</td>
<td>0.03</td>
</tr>
<tr>
<td>Peak vs. Non-peak</td>
<td>-0.005</td>
<td>Not sig.</td>
<td>0.109</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**dissection of spatial (lines and their architectural properties) and temporal (within-day) properties revealed variations**
Source Tracker gives proportion of contribution of each outdoor sample to the MTR line

For most of the lines, the corresponding outdoor location contributes the biggest

Mantel Test for correlation

Connectedness vs. phylogenetic distance $R = 0.47$, $p = 0.03$

Significant correlation between connectedness between lines and the similarity of their microbial communities
Taxonomic comparison

• Taxonomic comparison with:
  • NYC – 16S (Robertson et al. (2013) Appl Environ Microbiol 70: 3485-3493)
  • NYC – shotgun (Afshinnekoo et al. (2015) CELS in press)

<table>
<thead>
<tr>
<th>Rank</th>
<th>HK</th>
<th>NY (16S)</th>
<th>NY (shotgun)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Micrococcus</td>
<td>Staphylococcaceae</td>
<td>Pseudomonas stutzeri</td>
</tr>
<tr>
<td>2</td>
<td>Sphingobium</td>
<td>Moraxellaceae</td>
<td>Stenotrophomonas maltophilia</td>
</tr>
<tr>
<td>3</td>
<td>Enhydrobacter</td>
<td>Micrococcaceae</td>
<td>Enterobacter cloacae</td>
</tr>
<tr>
<td>4</td>
<td>Propionibacterium</td>
<td>Enterobacteriaceae</td>
<td>Acinetobacter radioresistans</td>
</tr>
<tr>
<td>5</td>
<td>Blastomonas</td>
<td>Corynebacteriaceae</td>
<td>Acinetobacter nosocomialis</td>
</tr>
<tr>
<td>6</td>
<td>Staphylococcus</td>
<td>Microbacteriaceae</td>
<td>Lysinibacillus sphaericus</td>
</tr>
</tbody>
</table>
Summary

- Common bacterial members: skin-associated OTUs
- From work, revealed that microbiome within the subway network is influenced by different factors: time, space, architectural (indoor/underground vs. outdoor/aboveground)
  - May be governed by nearby outdoor locations, and adjacent lines
Potential future work

Archived air samples (140)
- Shotgun metagenomics
- Fungi (ITS)

New samples
- New extensions (deepest)
- Surfaces
  - Skin microbiome of a HK/Asian cohort available (Leung et al, 2015, Sci Rep); baseline and longitudinal samples
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