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



Leung *et al.* (2014) *Appl. Environ. Microbiol.* 80: 6760-6770 Work done during summer 2013

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 nonpeak 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





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New lines

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



Sampling

4.32 m³

volume air per sample

= RIDE (platform + train sampling)

Integrated sampling/ Passengers' exposure

- 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

Overview



139 samples

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

Common genera

- Micrococcus (99.9% M. luteus)
- Sphingobium
- Enhydrobacter
- Proprionibacterium
- 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 a-diversity within a line

Exposure of regular commuters



abundance-unweighted and abundance-weighted UniFrac distances

	GROUP Comparisons	Global R (Unweighted)	P-value ^a	Global R (Weighted)	P-value ^a
Spatial/ Architectural	MTR vs. Outdoor	-0.023	Not sig.	0.046	Not sig.
	Outdoor vs. Outdoor	0.161	0.02	-0.003	Not sig.
	Line vs. Line	0.207	0.001	0.082	0.001
	Underground vs. Above-ground	0.232	0.001	0.064	0.004
Temporal	AM vs. PM	0.062	0.001	0.029	0.03
	Peak vs. Non-peak	-0.005	Not sig.	0.109	0.001

dissection of spatial (lines and their architectural properties) and temporal (within-day) properties revealed variations

Role of Outdoor + Adjacent Lines



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**

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)

Rank	НК	NY (16S)	NY (shotgun)
1	Micrococcus	Staphylococcaceae	Pseudomonas stutzeri
2	Sphingobium	Moraxellaceae	Stenotrophomonas maltophilia
3	Enhydrobacter	Micrococcaceae	Enterobacter cloacae
4	Propionibacterium	Enterobacteriaceae	Acinetobacter radioresistans
5	Blastomonas	Corynebacteriaceae	Acinetobacter nosocomialis
6	Staphylococcus	Microbacteriaceae	Lysinibacillus sphaericus

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

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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

3年

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