Exotic airborne bacteria identified in urban resuspended dust by next generation sequencing

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Abstract. The airborne transport of bacteria is a well-known phenomenon, making it possible to exchange species between ecosystems, but it also provides a tool for spreading of pathogenic microorganisms. As part of a large-scale study, microbial community of inhalable and respirable fractions (PM_{1-10}) of resuspended dust collected in Budapest (Hungary) has been characterised by culture-independent next generation sequencing (NGS) of variable 16S rRNA gene regions. Apart from common, mostly ubiqituos soil and organic material-dwelling bacteria, exotic airborne species have been identified, such as *Variovorax ginsengisoli*, previously isolated from Korean ginseng fields or *Exiguobacterium sibiricum*, isolated from the Siberian permafrost.

Keywords: urban resuspended dust; inhalable and respirable fractions; microbial community; next generation sequencing

1 Introduction

Each year, several billion tons of soil-derived dust enter the atmosphere, carrying microorganisms such as bacteria and fungal spores [1]. In exceptional cases they might travel distances over 5000 km [2]. Microorganisms can survive this transport [3], with the help of different mechanisms, especially against UV radiation [4].

Atmospheric dispersal, on one hand, is an important tool for exchange of bacteria between ecosystems [5, 6]. On the other hand, pathogenic bacteria might also be transported [7].

Airborne bacterial communities are especially frequently monitored during dust events [8]. While many dust-related studies use culture-based analyses [1], next generation sequencing has become a popular and accurate tool if whole communities are to be described [9, 10].

Most studies address the composition of the bacterial community from PM (Particulate Matter) samples [11]. PM itself poses high human health risk, moreover, the respirable fraction of PM ($PM_{2.5}$ and below) might act as a carrier for pathogenic bacteria (reviewed by Zhai et al., [12]).

In our study, microbial community of inhalable and respirable fractions of urban resuspended dust has been characterised by culture-independent next generation sequencing (NGS) of variable 16S rRNA gene regions. However, NGS revealed the presence of unique and exotic bacteria, which have most possibly been transported from other geographical regions and remained viable.

2 Materials and methods

2.1 Sampling

6 sampling locations were used in Budapest (Hungary). Sampling was completed on 9th March, 2013, at 6 locations (Figure 1). At each sampling site, sampling took 2 hours.

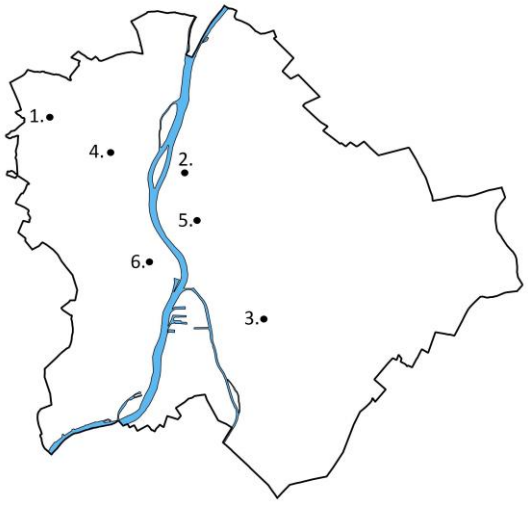

Fig. 1. Schematic map of Budapest, showing the sampling sites.

For sampling, a specific sampling device was used, developed by the Air Chemistry Group for collecting the inhalable and respirable fractions of resuspended road

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dust (PM_{10-1}) [13]. The instrument uses a leaf blower to mobilize dust from paved surfaces, simulating very windy conditions (wind speeds 100 km/h). The key unit in the apparatus is a PARTISOL-FRM model 2000 sampler that is operated at a flow rate of 16.7 L min-1 and contains a cyclone separator which collects the $PM_{(10-1)}$ fraction (particles with aerodynamically equivalent diameters between 10 µm and 1 µm) in bulk in a glass holder. The sampler is mounted on a mobile platform and powered with a portable electrical power generator.

2.2 DNA extraction and PCR amplification

DNA concentration was determined by using the Qubit dsDNA HS Assay Kit with the Qubit 2.0 Fluorometer according to the manufacturer's (LifeTechnologies) instructions.

For fusion method-based, unidirectional Ion Torrent bacterial 16S rRNA sequencing, we carried out PCR amplification using the forward primer consisting of the Ion Torrent adapter region (trP1: 5'-CCTCTCTATGGGCAGTCGGTGAT-3') fused to the 5' end of 16S rDNA target sequence (Bakt_341F: 5'-CCTACGGGNGGCWGCAG-3'). Triplicate reactions were carried out in 20 µl volumes by using KOD Hot Start DNA Polymerase (Novagen) according to the manufacturer's instructions, except that 10 ng of template gDNA and 3% DMSO were present in all reactions. Cycling conditions involved an initial 2 min denaturing step at 95 °C, followed by 25 cycles of 30 s at 95 °C, 30 s at 54 °C, and 30 s at 70 °C and a final elongation step of 5 min at 70 °C. PCR products were pooled and a two-step purification using 0.5 volumes of Agencourt AMPure XP Reagent was performed, respectively. The quality and quantity of the ca. 530 bp products were assayed by DNA 1000 Kit on Agilent Bioanalyzer 2100 instrument. 20 pM of total DNA were amplified in an emulsion PCR followed by target enrichment by using an Ion OneTouch 400 template kit, whilst sequencing of the pooled library was performed using an Ion Torrent PGM system and a 316v2 chip with the Ion Sequencing 400 kit according to the Life Technologies' protocol.

In order to classify reads covering the V3 and V4 regions of 16S rRNA gene up to species level, the bioinformatic pipeline described by Eiler et al., 2012 [14] was slightly modified. The operating system was Ubuntu, BioLinux 7 and the open-source softwares MOTHUR 1.31.2, R 3.0.1, Qiime 1.7.0, Cytoscape 2.7.0 and Krona excel template were applied. Out of 1.1 million total reads, sequences having an average quality number under 25, containing ambiguous bases, homopolimers longer than 8 bases, having more than 1 mismatch to the barcode sequence, more than 2 mismatches to the primer sequence or being shorter than 400 bp and chimeric sequences were discarded. The unique sequences were aligned to the appropriate reference small subunit databases (Greengenes, RDP and Silva). For operational taxonomic unit (OTU) calculations, a 97% similarity cutoff was used, and the OTU assignment data and sequence to sample mapping are used to generate the OTU-based table to count the number of sequences per OTU per sample.

3 Results and Discussion

Table 1 gives a summary of taxa diversity at the different locations and the abundant taxa are shown in Figure 2.

Table 1. Total number of taxa per site; number of taxa identified to Genus/Species level; and total read counts.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Total No. of Taxa	374	95	1441	961	171	906
Identified to Genus	241	69	954	654	117	580
Identified to Species	94	17	429	253	36	206
Total read count (RC)	1189	456	16186	8381	1738	23221

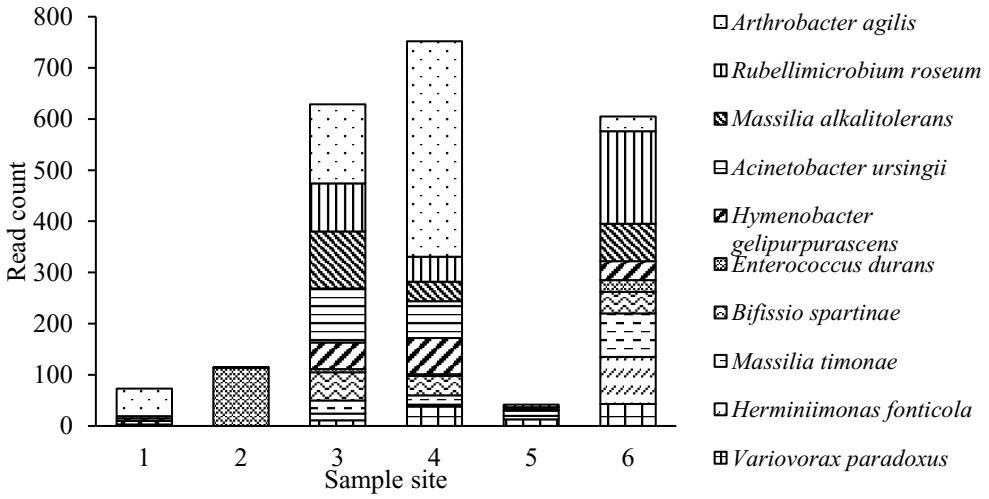

Fig. 2. Abundant species in the different sampling sites

In Sites 1, 3, 4 and 6 two species are prevalent: *Arthrobacter agilis* and *Rubellimicrobium roseum*. *A. agilis* is an ubiquitous soil-dwelling bacterium, in Hungary it has even been isolated from soda pans [15]. *R. roseum* is also a typical soil-dwelling species, isolated mostly from forest soils [16]. Though relatively few descriptive studies are available on urban soil bacterial community diversity, King in his review discusses that urban microbial flora somewhat resembles to natural soil flora but shows some level of disturbance [17].

Site 2 is dominated by *Enterococcus durans*. Similarly to other enterococci, this species has been reported from the gastrointestinal tract of humans and pets such as dogs or cats [18]. This site falls close to an urban park, most possibly dog walking is the main source of these bacteria.

No statistically significant correlation was found between sampling sites and the composition of the bacterial community based on Phyla (Read count by Site Kruskal-Wallis chi-squared = 4.0467, df = 5, p-value = 0.5427). Similarity of the sampling sites is shown in Figure 3.

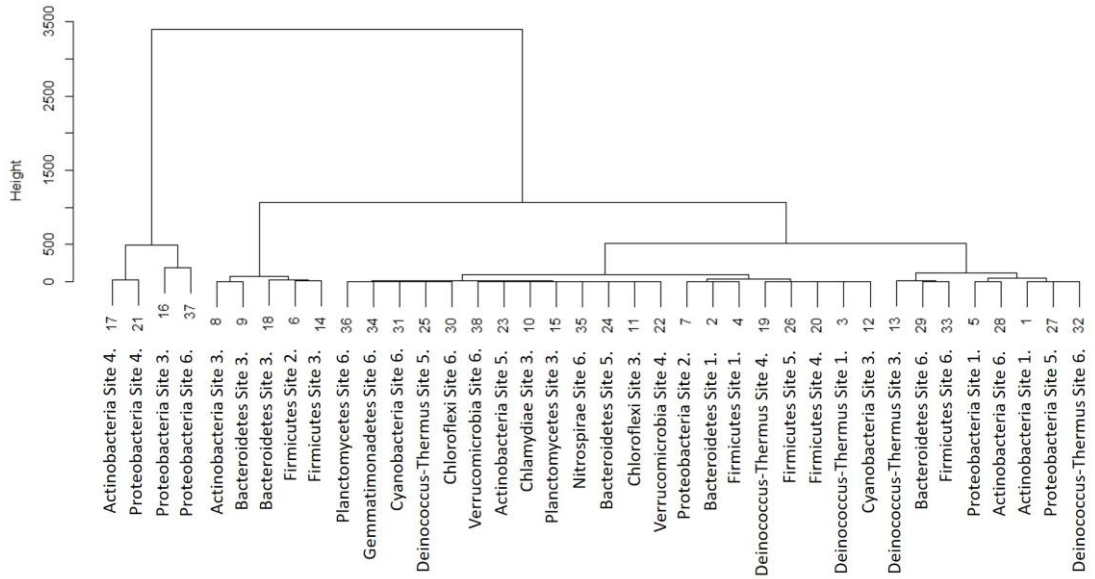

Fig. 3. Similarity of sampling sites based on Read Count per Phylum.

In our evaluation, those species have been considered exotic which fulfil the following criteria: (1) No data about their Hungarian occurrence is known; (2) Have been isolated in a geographically distinct area. In the following section, some examples are given. (Full species list is available upon request).

- *Deinococcus reticulitermitis*: Gram-negative, spherical, non-motile, non-sporulating bacterium, isolated from a termite gut [19]. Occurrence: Sample 1, read count (RC) 5; Sample 4, RC 2; Sample 6, RC 10.
- *Exiguobacterium sibiricum*: Gram-positive, rod-shaped, facultative aerobic, motile bacterium, isolated from Siberian permafrost [20]. Occurrence: Sample 3, RC 3.
- *Flavisolibacter ginsengisoli*: Gram-negative, aerobic, non-motile, rod-shaped bacterium, isolated from ginseng cultivating soil in Korea [21]. Occurrence: Sample 3, RC 4; Sample 4, RC 2; Sample 10, RC 7.
- *Mesorhizobium alhagi*: Gram-negative, rod-shaped, non-spore-forming, aerobic, motile bacterium, forming symbiotic root nodules on (Chinese) *Alhagi sparsifolia* [22]. Occurrence: Sample 1, RC 1; Sample 3, RC 1.
- *Variovorax ginsengisoli*: Gram-negative, aerobic or facultatively anaerobic, non-spore-forming, motile, rod-shaped bacterium, isolated from soil of a ginseng field [23]. Occurrence: Sample 3, RC 1; Sample 4, RC 1.

Of *Pseudomonas* genus, several potentially exotic species were identified:

- *P. koreensis*: Gram-negative, rod-shaped, bacterium, isolated from the Korean traditional fermented seafood jeotgal [24]. Occurrence: Sample 1, RC 2; Sample 3, RC 10; Sample 4, RC 4; Sample 6, RC 2.
- *P. xanthomarina*: Gram-negative, rod-shaped, motile bacterium, isolated from marine ascidians *Didemnum* sp. and *Halocynthia* sp. [25]. Occurrence: Sample 1, RC 3; Sample 3, RC 3; Sample 4, RC 5.
- *P. xinjiangensis*: Gram negative, motile bacterium, isolated from isolated from desert sand in Xinjiang province, China [26]. Occurrence: Sample 1, RC 2; Sample 3, RC 1; Sample 5, RC 1.

As in our study inhalable and resuspended dust was sampled, some of the species mentioned above might have distribution not restricted only to the region where

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they were isolated from, especially those isolated from soil such as *F. ginsengisoli*, *E. sibiricum* or *V. ginsengisoli*. On the contrary, those species which are specific in terms of occurrence, such as *D. reticulitermitis* which was isolated from a termite gut or *M. alhagi* which is a symbiont of *Alhagi sparsifolia* or *P. xanthomarina* which was isolated from marine ascidian (none of these co-existing taxa can be found in Hungary) most possibly can be regarded as of distinct origin.

Long-range transport of some of these above-mentioned species has been reported such as *Mesorhizobium* [27]. Deinococci were isolated in an Asian dust downwind area [28]. Species typically isolated from ginseng fields such as *Flavosolibacter and Pedobacter ginsengisoli* were detected on Asian dust particles collected during the Asian dust season from Beijing, China [29].

In addition to the general description of microbial community of resuspended dust, exotic species have been identified which are new to the Hungarian bacterial flora. It should be stressed, that in case of most of the taxa isolated from the samples, the spores remained viable according to culturing experiments.

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