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Metagenomic survey of bacterial diversity in the atmosphere of Mexico City using different sampling methods☆

N. Serrano-Silva, M.C. Calderón-Ezquerro*

Departamento de Ciencias Ambientales, Centro de Ciencias de la Atmósfera – Universidad Nacional Autónoma de México (UNAM), Circuito Exterior s/n, Coyoacan, University City, 04510 Mexico City, Mexico

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ABSTRACT

The identification of airborne bacteria has traditionally been performed by retrieval in culture media, but the bacterial diversity in the air is underestimated using this method because many bacteria are not readily cultured. Advances in DNA sequencing technology have produced a broad knowledge of genomics and metagenomics, which can greatly improve our ability to identify and study the diversity of airborne bacteria. However, researchers are facing several challenges, particularly the efficient retrieval of low-density microorganisms from the air and the lack of standardized protocols for sample collection and processing. In this study, we tested three methods for sampling bioaerosols — a Durham-type spore trap (Durham), a seven-day recording volumetric spore trap (HST), and a high-throughput 'Jet' spore and particle sampler (Jet) — and recovered metagenomic DNA for 16S rDNA sequencing. Samples were simultaneously collected with the three devices during one week, and the sequencing libraries were analyzed. A simple and efficient method for collecting bioaerosols and extracting good quality DNA for high-throughput sequencing was standardized. The Durham sampler collected preferentially *Cyanobacteria*, the HST *Actinobacteria*, *Proteobacteria* and *Firmicutes*, and the Jet mainly *Proteobacteria* and *Firmicutes*. The HST sampler collected the largest amount of airborne bacterial diversity. More experiments are necessary to select the right sampler, depending on study objectives, which may require monitoring and collecting specific airborne bacteria.

1. Introduction

Microbes such as bacteria are successful types of life on Earth because of their ability to adapt to new environments, reproduce quickly, and disperse globally. Their dispersion by wind as bioaerosols may be the most common way that microbes spread from land or oceanic sources, allowing them to overcome geographical barriers and disperse over long distances. Because of its chemical and physical characteristics (high solar radiation, limited nutrients and water availability, and large dispersal capability), the atmosphere appears be the most extreme environment for bacteria; however, it is documented that a fraction of these microorganisms are not only active metabolically under these conditions but also grow and reproduce (Burrows et al., 2009; Womack et al., 2010). Airborne bacteria represent a high risk not only for human public health, as pathogens or sources of allergenic components such as endotoxins (Gandolfi et al., 2013), but also for plants and animals. Furthermore, it is well known that the presence of bacteria in the atmosphere has important repercussions on the distribution of clouds and global precipitation, acting as ice nuclei and cloud condensation nuclei (Burrows et al., 2009; Zweifel et al., 2012).

The sources and sinks of airborne microorganisms have been reviewed, and it is estimated that the annual flux of bacteria through the atmosphere is 40–1800 billion grams (Burrows et al., 2009). This finding would explain the detection of bioaerosols in many different atmospheric environments, from urban centres to remote continental areas, and even in the mesosphere up to 77 kilometres (Smith et al., 2010).

Aerobiologists have probably undervalued the diversity of bioaerosols by using cultivation techniques in the laboratory; however, the use of molecular-based assays and the implementation of long-term atmospheric studies will generate more accurate regional abundance estimates (Smith et al., 2011). More recently, metagenomic studies on air quality, in which DNA is directly recovered from the samples without the need to grow microorganisms in agar

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* Corresponding author.
E-mail address: mclce@atmosfera.unam.mx (M.C. Calderón-Ezquerro).

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plates, have shown more information about the great diversity of airborne bacteria and highlighted our incomplete knowledge of global diversity (Be et al., 2015; Pignatelli et al., 2008; Yooseph et al., 2013).

Bioaerosols can be sampled in different ways, by active air samplers or by passive air sampling; there is a diversity of traditional and new devices to collect airborne microorganisms both culturable and not culturable. For a detailed description, see the review article of West and Kimber (2015). Recently, metagenomic studies on bioaerosols have been developed and contributed to our knowledge about bacterial diversity in the atmosphere. However, researchers are facing several challenges, particularly regarding the efficient retrieval of low-density microorganisms in the air and the lack of standardized protocols for sample collection and processing (Behzad et al., 2015; Jiang et al., 2015).

In this study, three methods for sampling bioaerosols were tested: the passive Durham-type spore trap, the Hirst-type spore trap (HST) and the high-throughput Jet spore and particle sampler. A protocol for metagenomic DNA extraction was standardized and optimized; universal molecular markers were used to detect bacteria, and the complete process was validated with deep sequencing in the Ion PGM™ machine (Ion Torrent, Thermo Scientific, USA) using the Metagenomics Ion 16S™ kit (Thermo Scientific, USA).

2. Methods

2.1. Sampling area

Samples were collected during one week from October 12th to October 18th in 2015, from the roof (15 m above the ground) of the Centre of Atmospheric Sciences Building (19°19′35″N, 99°10′34″W) in University City, the main campus of the National Autonomous University of Mexico, in the Coyoacan delegation of Mexico City. This site is one of the air quality monitoring stations of the Mexican Aerobiology Network (REMA), which is made up of 7 stations located within Mexico City, a megacity with extreme urban growth and serious environmental pollution (Calderón-Ezquerro et al., 2016).

2.2. Sample collection

Three different samplers were installed in monitoring station, allowing one meter of distance between them to avoid interferences. The samplers were working simultaneously during the sampling period:

- Durham-type spore trap

The Durham-type spore trap is a passive sampler that consists of a two-plate system that supports an adhesive surface horizontally exposed to capture particles that fall by sedimentation (Durham, 1946). The collection surface for the passive collection of bioaerosols consisted of a slide with 48 × 19 cm Melinex tape, prepared in sterile conditions inside UV light laminar flow hood cleaned with ethanol (70%). Before preparation, a paintbrush, a clamp and 5 g of Vaseline (Racel®, Mexico) were sterilized in an autoclave for 15 mins, then 25 ml of hexane (J. T. Baker®, USA) was added to the Vaseline and uniformly mixed. The mixed Vaseline:-hexane, slide and Melinex tape were exposed to UV light for 15 min. The Melinex tape was attached to the slide with a drop of sterile distilled water, and a thin layer of sterile Vaseline:hexane (1:5) mix was uniformly spread on the tape. The prepared slide was transported in a sterile box until it was installed in the Durham trap, and it remained in the trap for one week for sampling.

- Hirst-type spore trap (HST)

This equipment incorporates a rotating drum that moves clockwise. The sample is pulled in by a vacuum pump (airflow of 10 L/min), and bioaerosols are deposited by impaction onto cellophane tape (Melinex DuPont®, USA) that is adhered over the surface of the drum and covered with a thin layer of a mixture (1:5) of Vaseline and hexane (Calderón-Ezquerro et al., 2016). The drum was very carefully prepared in a sterile cabin with UV light to avoid contamination of the trapping surfaces. A mix of Vaseline:hexane (1:5) was prepared as described above. The drum was thoroughly cleaned with benzalkonium chloride (0.1%) and then irradiated with UV light, along with 35 cm of transparent plastic tape (Melinex tape), the mixed Vaseline:hexane, scissors and two dissector clamps to manipulate the tape. The trapping tape was attached to the drum, and the Vaseline was uniformly spread with a sterile paintbrush in a very thin layer. The drum was transported inside a sterile container provided by the manufacturer until it was installed in the HST. Before the installation of the drum, the HST was cleaned, verifying the intake orifice was free of dirt, and sprayed with benzalkonium chloride (0.1%). The trap was operated with a flux of 10 L/min air.

- High-throughput 'Jet' sampler

The 'Jet' sampler is a portable high-throughput trap where the sampled air is accelerated in a precision jet and forced against the orifice of a tube containing still air, which is connected with a hermetically sealed settling chamber in which the trapped particles fall under gravity and are evenly distributed on the base (http://www.burkard.co.uk/jetsamp.htm). In the normal configuration, the base of the chamber can hold an appropriate medium or detached leaf pieces of susceptible host plants, to be evaluated quantitatively. For this study, the 'Jet' sampler was adapted (modified) to capture in an Eppendorf tube the bioaerosols transported with dust. To do this, we installed an Eppendorf (1.5 ml) tube with a plastic funnel held with a cylinder inside the settling chamber. The device was operated with an air volume of 600 L/min. Before assembly, each part of the device was washed and sprayed with benzalkonium chloride (0.1%). Furthermore, the Eppendorf tube and plastic funnel were sterilized in an autoclave.

Immediately after the sampling period, samples of bioaerosols collected were preserved at −20 °C until extraction of metagenomic DNA.

2.3. Extraction, purification and quality of DNA

Samples were processed in a previously sterilized UV light laminar flow hood with benzalkonium chloride (0.1%). We use the following method to extract the metagenomic DNA from the bioaerosols collected: a mix of mechanical and chemical cellular lysis followed by recovery with phenol:chloroform and purification with magnetic beads. An extraction buffer was prepared with 0.1 M Tris-HCl pH 7.5, 0.05 M EDTA pH 8.0, 1 M KCl and 0.1% Nonidet P40. This buffer was sterilized by filtration through a Millipore 0.22 μm filter. To extract DNA from samples of air, the next protocol was established.

Bioaerosols recovered from the Melinex tape by sedimentation in the Durham-type spore trap were carefully placed in 2.0 ml screw-capped tubes containing 0.2 g acid-washed, sterile Ballotini beads and 200 μL of sterilized DNA extraction buffer plus 4 μL Proteinase K (20 mg/ml), and treated in the same way as the dust sample described below.

For samples taken with HST, the Melinex tape was removed from the drum in a sterilized cabin with UV light. The tape was cut

into seven pieces of 48 × 19 cm, and each one, corresponding to one day of sampling, was placed in a 2.0 ml screw-capped tube containing 0.2 g acid-washed, sterile Ballotini beads and 150 μL of sterilized DNA extraction buffer plus 2 μL Proteinase K (20 mg/ml). Lysis of the collected bioaerosols was done in a similar way to the dust sample described below, and 150 μL of phenol:-chloroform:isoamyl alcohol (25:24:1) was added to remove proteins and cellular debris. One hundred microliters of the aqueous phase were recovered, and DNA was recovered and purified with 180 μL of Agencourt AMPure® XP beads (Beckman Coulter, Inc) according to manufacturer instructions. The DNA was eluted in 15 μL of DNase-free water and stored at −80 °C until subsequent analysis.

Ten milligrams of the dust sample collected with the 'Jet' sampler were taken and placed in a 2.0 ml screw-capped tube containing 0.2 g acid-washed, sterile Ballotini beads and 200 μL of sterilized extraction buffer. Four μL of Proteinase K (20 mg/ml) (Thermo Fisher Scientific, Inc) was added and mixed by vortexing. The sample was processed in a FastPrep® (Thermo Electron, Corp) machine for two periods of 40 s at 4 m/s, cooling the tube on ice between runs. Four μL of RNase A (100 mg/ml) (Thermo Fisher Scientific, Inc) was added and mixed thoroughly by vortexing, and then the sample was incubated at 65 °C for 15 min, vortexing occasionally during incubation. Two hundred μL of phenol:chloroform:isoamyl alcohol (25:24:1) (Thermo Fisher Scientific, Inc) was added, and the sample was centrifuged for 15 min at 20 000 × g. The aqueous phase (150 μL) was recovered in a new Eppendorf tube and purified with 270 μL of Agencourt AMPure® XP beads (Beckman Coulter, Inc) according to manufacturer instructions. The DNA was eluted in 30 μL of DNase-free water and stored at −80 °C until subsequent analysis.

Negative controls for bioaerosol sampling were made for each sampler: a prepared slide for the Durham collector was placed in the equipment but not exposed, and the HST's drum and Jet's collection Eppendorf tube were left inside the equipment for 2 h with vacuum pumps off. After that, the Melinex tapes from the Durham and HST and the Eppendorf tube (1.5 ml) from Jet were processed through DNA extraction, and PCR for 16S rDNA genes was performed. No PCR products were obtained from the negative controls.

In total, nine DNA samples were obtained for this study. The integrity of the extracted DNA was verified by electrophoresis in agarose gel (1%). Furthermore, the purity of DNA was determined by measuring the ratio of the absorbance at 260 and 280 nm in a NanoDrop Lite Spectrophotometer (Thermo Scientific).

2.4. Library preparation and sequencing

Bacterial 16S rDNA genes were amplified by polymerase chain reaction (PCR) from 30 ng of DNA samples using the Ion 16S™ Metagenomics Kit, catalogue number A26216 (Thermo Fisher Scientific Inc.) using the Ion Torrent™ semiconductor sequencing workflow. The kit includes two primer sets that amplify the hypervariable regions V2-4-8 and V3-6,7–9, allowing 7 of 9 hypervariable regions (V2-3-4-6-7-8-9) of 16S rRNA gene from bacteria to be sequenced. The pooled, amplified fragments were ligated to Ion Adaptors to construct sequencing libraries. Nine libraries were prepared according to the manufacturer's instructions (https://tools.thermofisher.com/content/sfs/manuals/MAN0010799_Ion_16S_Metagenomics_UG.pdf) and sequenced on the Ion 318™ Chip v2 BC (Thermo Fisher Scientific, USA) with barcoded adaptors, using the Ion Torrent Personal Genome Machine™ (PGM™) System.

2.5. Data analysis

Data were analysed using the biological diversity suite of QIIME v1.9.1 (Caporaso et al., 2010), available in Python v2.7 repositories in the Ubuntu Gnome v16.10 distribution. The bam files from the Ion Torrent™ sequencing service were converted to fastq files, and base quality was checked in FastQC (Andrews, 2010). Reads with Phred scores lower than Q25 in the outer sections were discarded using Trimmomatic SE (Bolger et al., 2014). Chimeric sequences were removed given <85% similarity with reference database (Greengenes v13.8). OTU picking was done at 90% similarity with uclust (Edgar, 2010) and at least 10 identical sequences; the representative phylogenetic OTUs (phylotypes) were assigned using the RDP classifier (Wang et al., 2007) and Greengenes v13.8 reference database. Microbial diversity for each sample (alpha_diversity.py) was determined by calculating the Shannon and Simpson indices, Chao1 and Observed species from a multiple rarefaction. To compare the microbial diversity between samples, qualitative (unweighted UniFrac) and quantitative distances (weighted UniFrac) were calculated.

Libraries constructed from the Hirst-type spore trap (HST) in Coyoacan were grouped and treated as a single sample to be analyzed with libraries from the other samplers (Durham and Jet). Pie charts showing abundance were plotted using Krona Tools v2.7 (Ondov et al., 2011). Significant differences between the abundances of assigned phylotypes were established with G-test (log-likelihood ratio g_test).

2.6. Nucleotide sequence accession numbers

The nucleotide sequences of bacterial 16S rDNA gene fragments have been deposited at the NCBI Sequence Read Archive under accession numbers SAMN07346063 (CU_HST12), SAMN07346078 (CU_HST13), SAMN07346106 (CU_HST14), SAMN07346145 (CU_HST15), SAMN07346149 (CU_HST16), SAMN07346152 (CU_HST17), SAMN07346153 (CU_HST18), SAMN07346270 (CU_DURHAM), SAMN07346271 (CU_JET).

3. Results

3.1. Metagenomic extraction of DNA

Good DNA purity was obtained using the previously described DNA extraction method and proved suitable for downstream processing, i.e., ribosomal gene PCR. For bioaerosols collected with the Durham-type spore trap, we obtained on average 15.35 ng/μL of DNA (average of 3 measurements in Nanodrop) with good purity (absorbance ratio $A_{260/280}$ 1.79). Samples collected with the Hirst-type spore trap (HST) were processed by collection day, and yields of DNA were between 10.33 and 10.67 ng/μL of DNA with absorbance ratios $A_{260/280}$ between 1.47 and 1.76. A good quantity and purity (20.50 ng/μL and absorbance ratio $A_{260/280}$ 1.56) of DNA from bioaerosols collected in dust with the high-throughput 'Jet' sampler was also obtained.

3.2. Assessing bacterial airborne diversity by sampling method

A total of 753 417 reads were obtained from the nine sequenced samples, with a minimum of 64 191 and a maximum of 140 162 reads per sample (Table 1). 54% of total reads were valid after quality filtering and removal of chimaeras for subsequent analysis. Sequences retrieved from the 9 libraries were grouped based on > 97% sequence identity, resulting in 7122 bacterial phylotypes. A high number of unique phylotypes were found (Table 1).

The bacterial diversity of the bioaerosols collected in Coyoacan

Table 1
Number of raw 16S rDNA gene amplicon reads and bacterial phylotypes defined to 90% identity.

Sample	Total reads	Non-chimeric sequences	Number of bacterial phylotypes	Number of unique phylotypes
Durham	79434	49919	4131	1357
HST12	85843	48114	5652	1432
HST13	140062	69875	6102	1072
HST14	69207	28497	4718	1652
HST15	64191	35903	5209	1640
HST16	82510	37567	5342	1576
HST17	85843	45843	5562	1394
HST18	70456	38131	5283	1547
Jet	75871	55697	2577	885

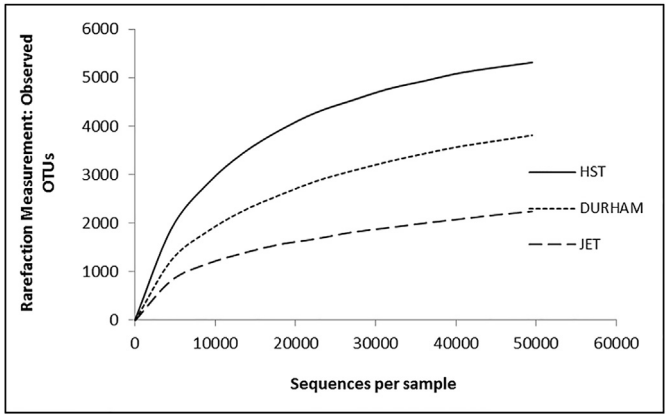

Fig. 1. Rarefaction curves of observed phylotypes (OTUs) in 16S rDNA libraries from airborne bacteria collected with Durham, HST and Jet samplers.

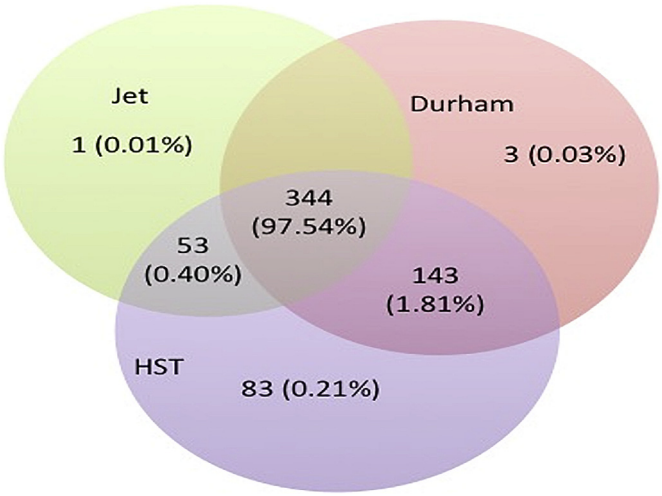

Fig. 2. Number of bacterial airborne genera collected with Durham, HST and Jet samplers. Overlapping genera between samplers and sequence abundances (in brackets) are shown.

with Durham, HST and Jet was assessed using several parameters, including rarefaction curves and diversity indexes (Fig. 1, Table 2). We consider that the sampling depth with three samplers was adequate to accurately characterize the airborne bacteria since slope of the rarefaction curves became nearly asymptotic (Fig. 1). Rarefaction curves of the number of observed phylotypes and richness estimator Chao1 for each sample indicates that the recovered libraries from the Hirst-type spore trap (HST) had greater species richness and abundance (Supplementary Fig. 1S), being bacterial communities more heterogeneous than those collected with the Durham and Jet samplers.

Including all 3 sampling methods in Coyoacán, 6042 bacterial phylotypes (grouped based on > 97% sequence identity) were retrieved. A Venn diagram of bacterial genera found with each of the three samplers, Durham, HST and Jet, was drawn (Fig. 2). Overall, 627 genera of airborne bacteria from Mexico City were collected. While 344 of the 627 (55%) were trapped with all three samplers, 623 could be detected with HST, 490 with Durham and 398 with Jet (Fig. 2).

3.3. Taxonomic analysis

Globally, a high proportion of sequences (9.6%) recovered with the three samplers used in this study to collect bioaerosols corresponded to chloroplasts (16S rRNA encoding gene from plants) assigned to phylum *Cyanobacteria*. Those sequences were mainly attributed to *Streptophyta* (8.7%) with a low proportion of *Chlorophyta* and *Stramenopiles* (<1%). The relative abundances from each sampler were as follows: Hirst-type spore trap – HST, 18.1%; Durham spore trap, 10.7%; and high-throughput 'Jet' sampler, 0.6%.

As shown in Figs. 3 and 4, a great number of Cyanobacteria were collected with the Durham passive spore trap (44.6%) in Coyoacan, Mexico City. These *Cyanobacteria* were mainly classes *Nostocophycideae* (19%), *Oscillatoriophycideae* (18%) and *Synechococcophycideae* (4%), and other *Cyanobacteria* found in minor quantities were *Gloeobacterophycideae* and *ML635J*-21 (<1%).

Proteobacteria, *Actinobacteria* and *Firmicutes* were mainly collected with HST (Figs. 3 and 5). The diversity and abundance of airborne bacteria collected with HST in Mexico City (Coyoacan) were shown to be highly similar during the seven days of sampling (Supplementary Figs. S1 and S2).

We found that the Jet sampler mainly collected *Proteobacteria* (70.5%) and *Firmicutes* (19.7%) (Figs. 3 and 6), while the Durham sampler, in addition to Cyanobacteria (45.0%), mainly collected *Proteobacteria* (25.6%), *Actinobacteria* (20.9%) and *Firmicutes* (5.0%) (Figs. 3 and 4). Globally with the three samplers, *Bacteroidetes* was found in less than 5% of each sample and other phyla such as

Table 2
Microbial richness and diversity of 16S rDNA libraries based on 90% identity OTUs from airborne bacteria collected with Durham, HST and Jet samplers.

Sampling method	Richness estimator Chao1	Shannon diversity index H	Simpson diversity index D
Durham-type spore trap	4713.12	8.94	0.990
Hirst-type spore trap	5824.04	10.58	0.997
Jet spore and particle sampler	3260.07	8.18	0.988

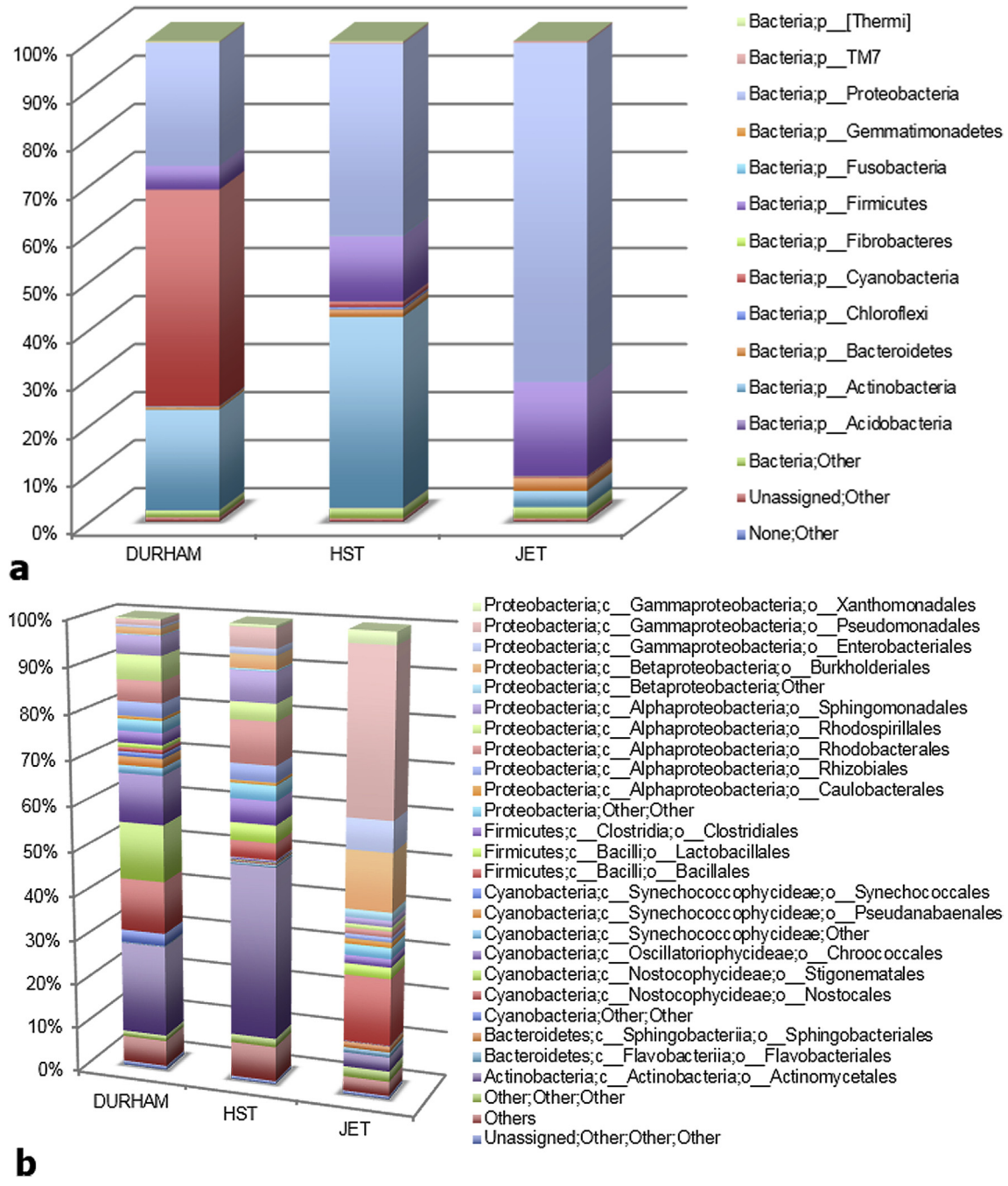

Fig. 3. Relative abundance of airborne bacteria from Mexico City at level of (a) phylum and (b) order, in samples collected in Coyoacan, Mexico City with Durham-type spore trap (DURHAM), Hirst-type spore trap (HST) and Jet spore and particle samplers (JET). Orders with more than 1% are shown.

Acidobacteria, *Chloroflexi*, *Fusobacteria*, *TM7* and *Thermi* were found in proportions of less than 1% for each sampler. Phyla *Fibrobacteres* and *Gemmatimonadetes* were not detected using Durham.

Alphaproteobacteria (*Rhodobacteraceae*, *Acetobacteraceae* and *Sphingomonadaceae* families) and *Actinobacteria* (*Micrococcaceae* family) were caught in high proportions with the HST sampler (2–9%) (Fig. 5) and the Durham sampler (4–5%), in addition to *Cyanobacteria* with Durham (Fig. 4). *Gammaproteobacteria* (*Enterobacteriaceae*, *Moraxellaceae* and *Pseudomonadaceae* families; 7–22%), *Bacilli* (*Exiguobacteraceae*; 12%) and *Betaproteobacteria* (*Oxalobacteraceae*; 12%) were the most abundant bacterial classes attached to dust particles collected with the Jet sampler (Fig. 6). The most abundant genera collected with the three bioaerosol samplers (Durham, HST and Jet) are shown in Table 3.

Significant differences (P value and FDR < 0.05) between abundances of assigned bacterial genera were established with a G-test (Supplementary Table 2S). Taxa associated with the Jet sampler in addition to those above: *Enterobacteriaceae*, *Moraxellaceae, Pseudomonadaceae, Exiguobacteraceae* and *Oxalobacteraceae,* included *Xanthomonadaceae* (2%) from *Gammaproteobacteria.* Interestingly, 83% of *Cyanobacteria* were strongly associated with the Durham sampler and 74% of assigned phylotypes at the genus level were strongly associated with the Hirst-type spore trap.

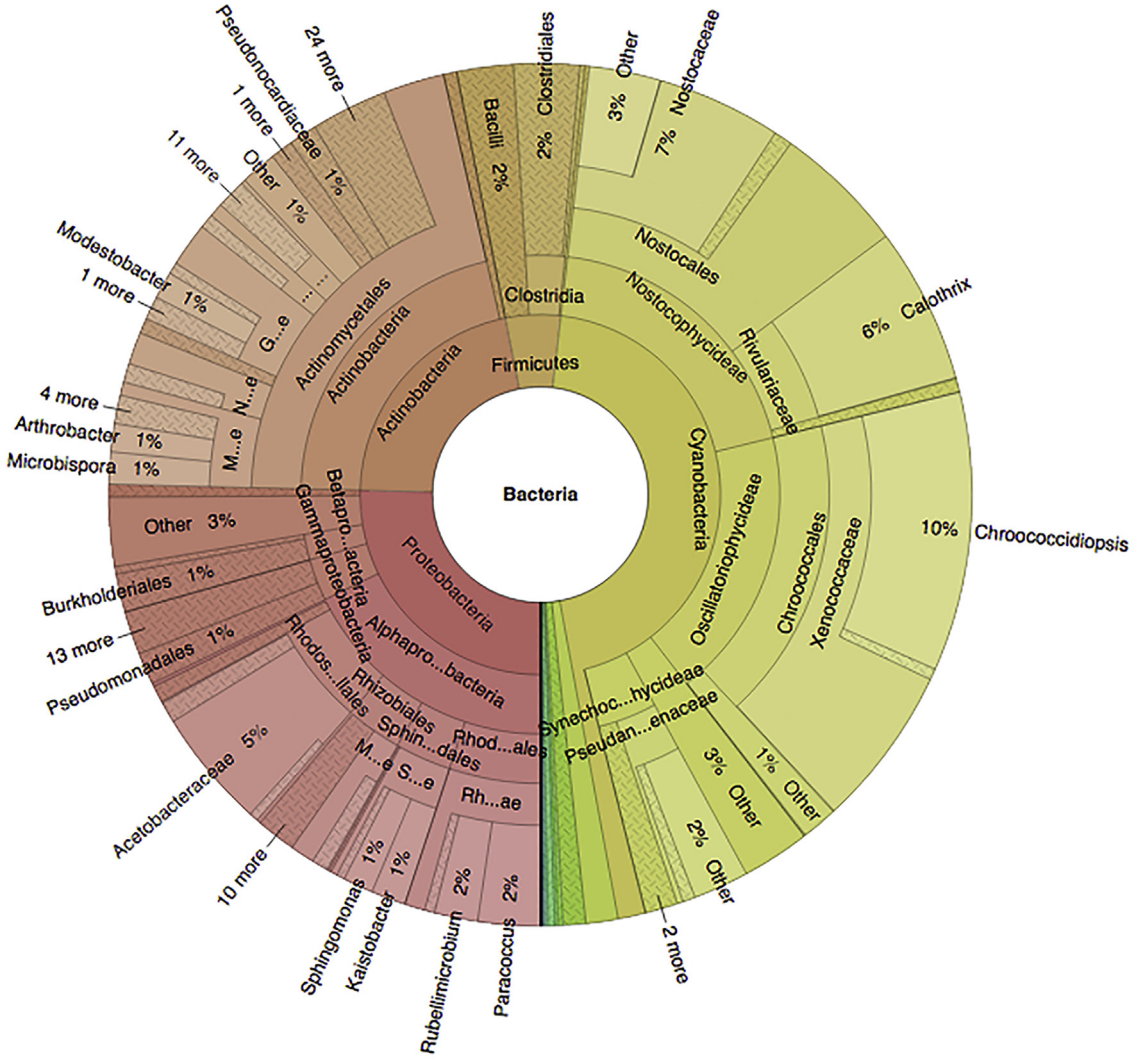

Fig. 4. Taxonomic distribution of airborne bacteria in Coyoacan, Mexico City, collected with Durham-type spore trap.

4. Discussion

Studies of microbial communities in the air with metagenomic sequencing have been challenging, mainly because of the low amount of particulate matter collected in the air and the low biological content of the samples (Jiang et al., 2015). Besides this, another air pollutants such as black carbon (especially in megacities with extreme urban growth like Mexico City) can be washed down in the DNA extraction process and its elimination is difficult. The use of kits for DNA extraction is a good option, however the yield of the obtained metagenomic DNA decreases with these. With the used samplers low amount of airborne particles are collected, however we were able to obtain metagenomic DNA of airborne biological particles, in a good quality and in sufficient quantity to be amplified by PCR.

To test if three samplers of bioaerosols, that extensively have been used for pollen monitoring, can be used to sample the diversity of airborne bacterial communities, we analysed libraries from three samplers working simultaneously in a monitoring station (University City in the Coyoacan delegation) during one week.

The Durham spore trap is a simple gravity-based method of sampling, which works by passive deposition of particles onto a surface (Durham, 1946) and can be useful in sites without dominant wind or high turbulence (West and Kimber, 2015). Our results show that the Durham is a good sampler for collecting mainly airborne *Cyanobacteria*. Most of *Cyanobacteria* found in this study such as Nostocales and Stigonematales from *Nostocophycideae* class as well as Oscillatoriales from *Oscillatoriophycideae* class, are filamentous *Cyanobacteria*, with sizes greater than 20 μm (Flores and Herrero, 2010). Given that the Durham-type passive spore trap collects particles that fall down by sedimentation, *Cyanobacteria* were the most favoured, very likely because of their large size.

The presence of Cyanobacteria (or blue-green algae) in the atmosphere is not strange; more than 350 morphological taxa (genera or species) have been identified in aerobiological studies (Genitsaris et al., 2011), including in the southern metropolitan area of Mexico City (Roy-Ocotla and Carrera, 1993). Studies of atmospheric Cyanobacteria are scarce; for Mexico City, just one study has been published (Roy Ocotla and Carrera, 1993). It reported six cyanobacterial species that to date are grouped in *Chroococcales*, *Oscillatoriales, Nostocales* and *Pleurocapsales*. *Cyanobacteria* species have several morphological forms that are genetically determined but can change in response to extreme environmental conditions, so they adapt to their surroundings to support optimal survival (Singh and Montgomery, 2011; Vincent, 2009). Such adaptations include production of osmolytes and water-retaining mucilaginous

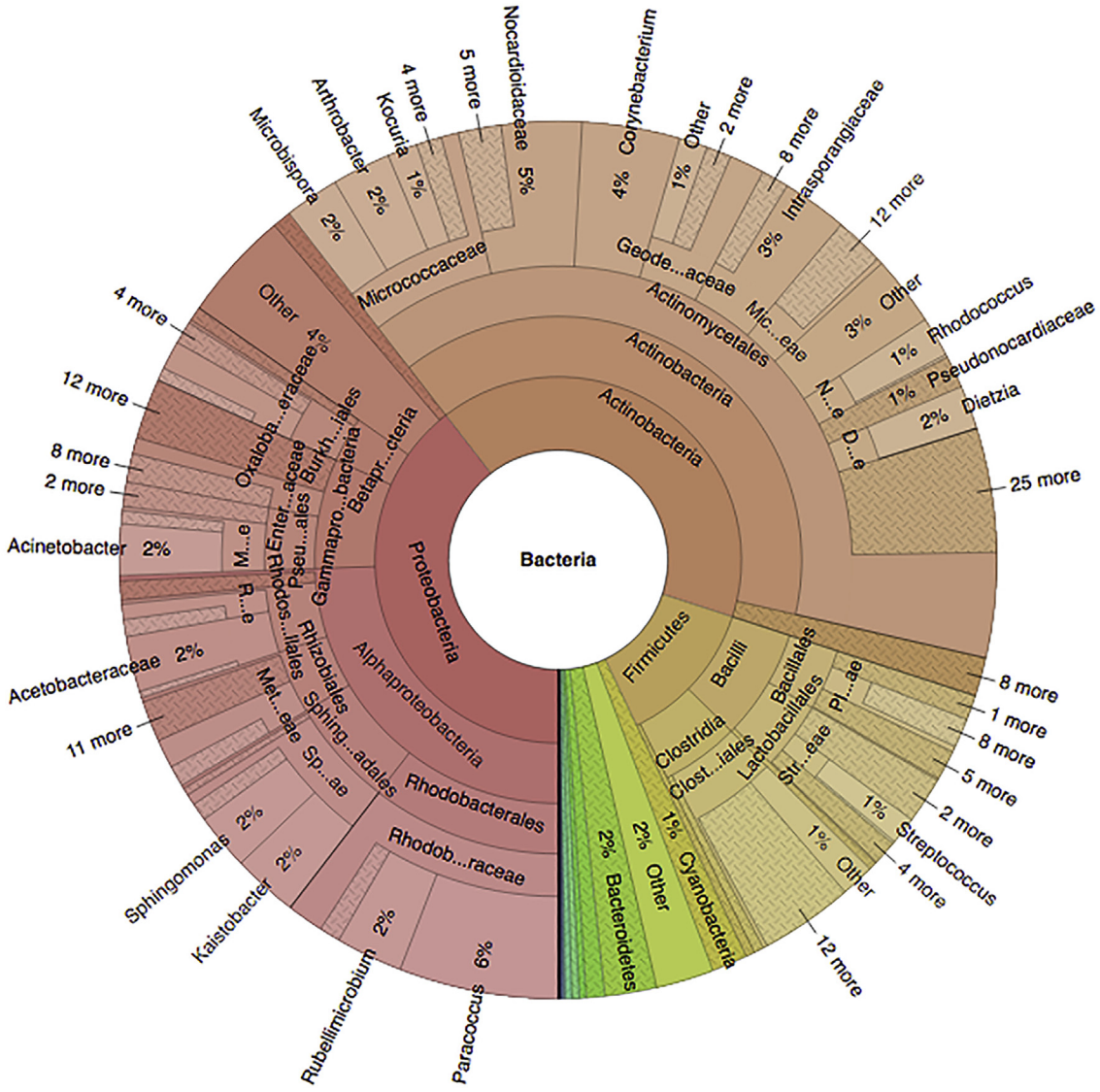

Fig. 5. Taxonomic distribution of airborne bacteria in Coyoacan, Mexico City, collected with Hirst-type spore trap.

sheaths for desiccation or salinity tolerance, production of heat-shock proteins for thermal tolerance, and synthesis of both intracellular and extracellular UV-screening compounds or UV-shock proteins to reduce the negative effects of UV exposure (Dillon et al., 2002).

The HST (Hirst-type Spore Trap) is used in many countries, mainly for counting daily pollen and/or studying fungal spores by microscopic viewing. The present is one of the first studies in that HST has been used to sample airborne bacteria, recently the use of HST was evaluated for monitoring of bacteria in urban air samples (Núñez et al., 2017). Our results show that HST is a useful and robust device for trapping a great diversity of airborne bacteria (Table 2, Supplementary Figs. 1S and 2S), along with pollen grains and fungal spores (Núñez et al., 2017; West and Kimber, 2015). *Proteobacteria*, *Actinobacteria* and *Firmicutes* were mainly collected with HST, as we could see during the seven days of sampling (HST12-HST18) (Supplementary Figs. 1S and 2S). In addition to the advantage HST provides of separately analysing data for each day, the device recovered a greater species richness and abundance of airborne bacteria than the Durham and Jet samplers (Fig. 1, Table 2). The greater diversity of bacteria in the air collected with HST in the sampled period could be influenced by the depth of the sequencing since these samples were processed for DNA extraction and preparation of 16S libraries per day and then collapsed and analysed by week (as samples collected with Durham and Jet).

The lower diversity of bacteria were collected with the Jet sampler than with Durham or HST (Fig. 1). Previous studies have reported the choice of sampling device may affect the composition of the sampled community (Fahlgren et al., 2011; Hoisington et al., 2014; Pasquarella et al., 2000). Sampler Jet, due to the high suction (600 L/min), collects a large pool of inorganic and organic particles that can influence the DNA recovery, so more studies are necessary with this device. Our results shown Jet sampler was a good device for collecting mainly airborne *Proteobacteria* (Fig. 3, Table 3).

The most abundant airborne bacterial phyla in monitored samples from Mexico City were *Proteobacteria* and *Actinobacteria* (Supplementary Fig. 2S). A comparable study in New York City, USA, reported *Proteobacteria* as the most dominant phylum (52%) in outdoor sampling (Yooseph et al., 2013). Other very important detected phyla were *Firmicutes*, *Cyanobacteria* and *Bacteroidetes* (in a minor proportion), but the proportion of these with each sampler (HST, Durham and Jet) was variable (Fig. 3, Table 3), indicating that

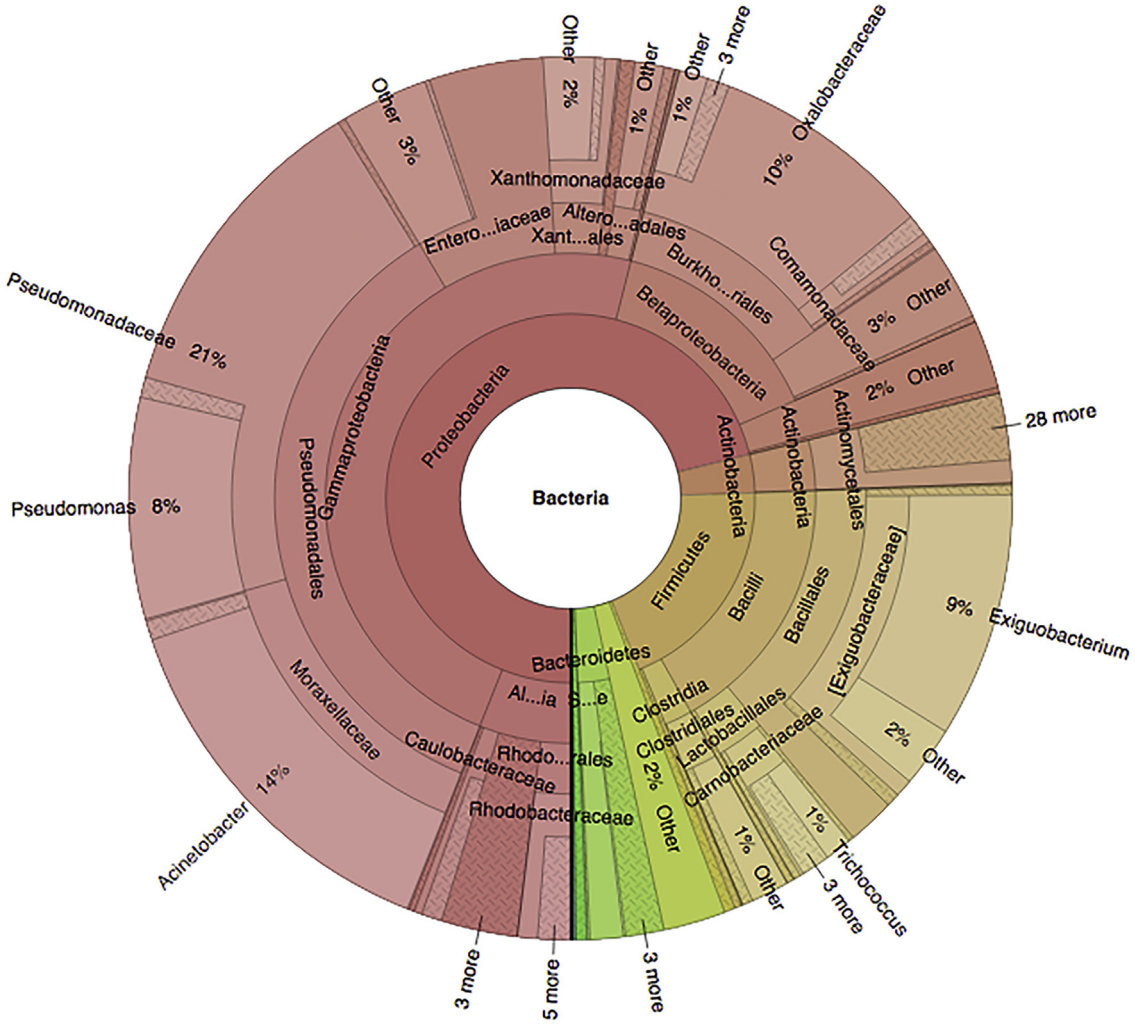

Fig. 6. Taxonomic distribution of airborne bacteria in Coyoacan, Mexico City, collected with high-throughput 'Jet' spore and particle sampler.

Table 3
Descriptions and relative abundances of the main airborne bacterial genera collected with samplers J (Jet spore and particle sampler), HST (Hirst-type spore trap) and D (Durham-type spore trap) from Coyoacan, Mexico City.

Phylum	Class	Genus	Sampler	Relative abundance by library[a]
Proteobacteria	*Gammaproteobacteria*	*Acinetobacter*	J, HST, D	13.7%, 2.8%, 0.7%
Firmicutes	*Bacilli*	*Exiguobacterium*	J, HST, D	8.9%, 1.0%, 0.2%
Proteobacteria	*Gammaproteobacteria*	*Pseudomonas*	J, HST, D	8.1%, 0.9%, 0.2%
Cyanobacteria	*Oscillatoriophycideae*	*Chroococcidiopsis*	D, HST, J	10.3%, 0.2%, Tr
Cyanobacteria	*Nostocophycideae*	*Calothrix*	D, HST, J	5.8%, 0.1%, Tr
Proteobacteria	*Alphaproteobacteria*	*Paracoccus*	HST, D, J	5.3%, 2.3%, 1.0%
Actinobacteria	*Actinobacteria*	*Corynebacterium*	HST, D, J	4.1%, 0.6%, 0.1%
Actinobacteria	*Actinobacteria*	*Arthrobacter*	HST, D, J	2.2%, 1.0%, 0.1%
Proteobacteria	*Alphaproteobacteria*	*Kaistobacter*	HST, D, J	2.2%, 1.3%, 0.2%
Proteobacteria	*Alphaproteobacteria*	*Rubellimicrobium*	HST, D, J	2.0%, 1.6%, 0.1%
Actinobacteria	*Actinobacteria*	*Microbispora*	HST, D, J	1.9%, 1.2%, 0.3%
Proteobacteria	*Alphaproteobacteria*	*Sphingomonas*	HST, D, J	1.8%, 1.1%, Tr
Actinobacteria	*Actinobacteria*	*Rhodococcus*	HST, D, J	1.6%, 0.8%, Tr
Actinobacteria	*Actinobacteria*	*Dietzia*	HST, D, J	1.5%, 0.3%, Tr
Actinobacteria	*Actinobacteria*	*Kocuria*	HST, D, J	1.2%, 0.6%, Tr
Firmicutes	*Bacilli*	*Lactobacillus*	HST, D, J	1.1%, 0.2%, Tr
Firmicutes	*Bacilli*	*Staphylococcus*	HST, D, J	0.9%, 0.1%, 0.1%
Actinobacteria	*Actinobacteria*	*Modestobacter*	D, HST, J	1.0%, 0.5%, Tr
Cyanobacteria	*Synechococcophycideae*	*Acaryochloris*	D, HST, J	0.9%, Tr, Tr
Bacteroidetes	*Flavobacteriia*	*Chryseobacterium*	J, HST, D	1.0%, 0.4%, Tr
Firmicutes	*Bacilli*	*Trichococcus*	J, HST, D	1.0%, 0.2%, 0.1%
Firmicutes	*Bacilli*	*Desemzia*	J, HST, D	1.0%, Tr, Tr

[a] Tr means relative abundances lower than 0.1% from total sequences by library.

the structure of airborne bacterial communities was affected by the device used. Using the passive sedimentation method on culture media, as well as massive sequencing to describe the airborne bacterial diversity of Mexico City, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* were the most abundant phyla found by García-Mena et al. (2016).

The genera *Acinetobacter*, *Exiguobacterium* and *Pseudomonas*, collected in high proportion mainly with the Jet sampler (Table 3), were found to be highly abundant by García-Mena et al. (2016) during summer and winter in the lower atmosphere of Mexico City. It is reported that epiphytic microbes are potential contributors to atmospheric microbiota and that their concentration can be from five-to tenfold higher near plants than away from such sources (Lymperopoulou et al., 2016). The selection of bacterial taxa by each sampler, in addition to the particle size, seemed to be related to the presence of abundant vegetation near the locations of the samplers, as well as other sources as soil and skin microbiota. For example, *Acinetobacter* (*Alphaproteobacteria*), which consists of strictly aerobic, Gram-negative coccobacilli that can use a variety of organic materials as sources of carbon, is found in soils and in living organisms, among other locations. *Pseudomonas* (*Gammaproteobacteria*), which consists of rod-shaped, aerobic, Gram-negative bacteria, is found widely in soils, water, and plants. Some species of *Paracoccus* (*Rhodobacteraceae*), small Gram-negative coccoids or coccobacilli, can grow as methylotrophs on one-carbon compounds. *Corynebacterium* (*Actinobacteria*; facultatively anaerobic, Gram-positive bacilli) and *Staphylococcus* (*Firmicutes*; Gram-positive cocci) are part of the normal saprophytic flora of human skin. Some bacteria recovered are well documented for their capability to tolerate high levels of UV radiation (*Exiguobacterium*), and thermophiles (*Rubellimicrobium* and *Microbispora*) were found in high proportions as well. However, samples from more sampling locations would better support this hypothesis.

Some species of *Acinetobacter* and *Pseudomonas* are important as risks to human health. *Acinetobacter* has been found to contaminate respirators and hospital air, being a potential risk mainly for immunocompromised patients. *Pseudomonas* is not usually a cause of infections in healthy people, but compromised patients have a high risk of infections in the lungs or skin.

Our data support the hypothesis that the sampling device influences the composition of the sampled community, as Durham, HST and Jet preferably collected different bacterial groups. However, the bacterial genera shared between the samplers were the dominant organisms in the air; 97.54% of total sequences recovered in this study were assigned to 55% of the genera trapped with all three sampling methods (Fig. 2). Although HST was shown to be a robust device for collecting the bacterial community in the air of Mexico City, Durham and Jet trapped some different airborne bacteria. For example, *Sodalis* (*Gammaproteobacteria*, *Enterobacteriales*) could be collected only with the Jet sampler, and some *Cyanobacteria* (*Hydrocoleum, Anabaena* and some *Rivulariaceae*) were collected only with the Durham sampler.

We conclude that we were able to standardize a methodology to collect bioaerosols and extract good quality DNA for high-throughput sequencing; three sampling methods to detect airborne bacteria were used and analysed (Durham, Hirst and Jet spore traps), displaying a convenient method for using them to determine the diversity of bacterial outdoor bioaerosols. The Hirst-type spore trap HST collected the highest amount of this diversity, but the Durham-type spore trap and the Jet spore and particle collector preferentially collected some bacterial groups (*Cyanobacteria* and *Proteobacteria,* respectively). Depending on study objectives, a suitable sampler should be selected to monitor and collect the relevant bacteria from the air. Because atmospheric microbiota changes in depending on meteorological conditions, more experiments are necessary using these devices to conclude the best sampler for monitoring airborne bacteria. It is important to note that daily recovery of DNA from airborne bacteria is sometimes difficult, especially when bioaerosols have decreased due to heavy rains, and in that case we recommend pooling samples collected with Durham, Hirst and Jet spore traps by the week for higher metagenomic DNA yield.

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Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.12.035.

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