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

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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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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