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Variability of airborne bacteria in an urban Mediterranean area (Thessaloniki, Greece)

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HIGHLIGHTS

- Airborne bacterial abundance, biomass and composition were studied in Thessaloniki.
- The highest values of bacterial abundance were recorded during summer.
- No significant seasonal differences were found between summer and winter.
- Air temperature was found to significantly affect the airborne bacterial community.
- The majority of OTUs were affiliated to taxa derived from soil and wastewater.

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ABSTRACT

The abundance, biomass and the taxonomic composition of the total airborne bacterial communities in a coastal urban area of Northeastern Mediterranean Sea were examined. In total, 27 air samples were collected across three seasons from a sampling point of approximately 30 m altitude in the center of the city. The abundance and biomass were determined with the use of epifluorescent microscopy, while the taxonomic composition was characterized by next-generation sequencing methods. Overall, the highest values of bacterial abundance were recorded during summer, with values exceeding abundances recorded in other urban sites across Europe, reaching 41×10^4 cells m^{-3}. Out of 6 core meteorological parameters, only air temperature was found to significantly affect the abundance and biomass of airborne bacteria. Concerning the taxonomic composition of the airborne bacterial community, the group of Proteobacteria was the most diverse, with 47% of the total number of OTUs belonging to them, followed by Firmicutes, Actinobacteria and Bacteroidetes. The most dominant OTU belonged to γ-Proteobacteria, and was closely affiliated to *Pseudomonas* sp., a taxon commonly found to actively participate in the formation of ice-nuclei in the atmosphere. Finally, 19 OTUs were shared between all seasons and were found to be among the most dominant overall. The majority of these OTUs were affiliated to genera from soil and wastewater origin, while several were affiliated to genera that include known or opportunistic pathogens. Yet, only rare OTUs were affiliated to taxa with possible marine origin (e.g. *Synechococcus* sp.). The results showed that the atmosphere of the study area harbors a diverse and abundant bacterial community.

1. Introduction

The atmosphere is now broadly accepted to be a habitat of abundant and variable microbial communities (see Lighthart, 2000 for a review). Particularly, airborne bacteria may reach abundances that exceed hundreds of thousands of cells per m^3 (Lighthart, 1997) and consist of a wide range of taxa in urban environments (Brodie et al., 2007; Bowers et al., 2011a). These bacterial communities have been associated with various processes, being causative agents of human health conditions (Douwes et al., 2003), and even

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influencing atmospheric conditions (e.g. Christner et al., 2008; Bowers et al., 2009). Despite many decades of research on the topic, little is known about the diversity and variability of airborne bacterial communities in urban areas, in comparison to the vast information about the physical and chemical characteristics of the atmosphere (Bowers et al., 2013).

Until now, most studies have focused only to the culturable fraction of the airborne bacterial communities (e.g. Shaffer and Lighthart, 1997; Fang et al., 2007; Ravva et al., 2012; Dueker et al., 2012), which is a large underestimation of the total bacterial diversity (Torsvik et al., 2002). The recent advances in high-throughput sequencing can offer unprecedented insights by revealing a surprising diversity of previously undetected and yet-uncultured bacteria (Shokralla et al., 2012). Next-generation sequencing (NGS) methodologies are already commonly used in the characterization of temporal and spatial variability of microbial communities in various environments (e.g. marine; Gilbert et al., 2012; freshwater; Staley et al., 2013; soil; Roesch et al., 2007; gut; Kormas et al., 2014, extreme Kormas et al., 2015, and various other environments; see Shokralla et al., 2012 for a review). Following the increasing use of NGS to explore environmental microbial diversity in several different ecosystems, few recent aerobiological studies have attempted to investigate the airborne bacterial communities in urban areas (e.g. Bowers et al., 2011a; Bertolini et al., 2013; Woo et al., 2013; and for reviews see also Gandolfi et al., 2013; Behzad et al., 2015 and references therein). These studies resulted in an increased level of understanding of the bacteria found in the atmosphere, their dynamics and the effect of various abiotic conditions on their variability.

In the present study, we attempted to investigate the abundance and biomass of airborne bacterial communities as well as their taxonomic composition with the use of NGS, for a coastal urban area of the Northeastern Mediterranean Sea across three seasons. The questions we aimed to address were: (1) How does the abundance and biomass of the airborne bacteria differ between seasons? (2) How diverse is the airborne bacterial community? and (3) How might certain meteorological conditions affect the abundance, biomass and taxonomic composition of the airborne bacterial communities? The study area was Thessaloniki City, which is situated at the northernmost tip of Thermaikos Bay, Northern Greece. Thessaloniki is a densely populated and highly polluted industrial city (Samara et al., 2003). The climate of the city is typically Mediterranean, with hot and dry summers and mild and wet winters (Giannaros and Melas, 2012). The summer period is characterized by high temperature, low relative humidity and precipitation, while the cold period experiences a rise in relative humidity, accompanied by a drop in temperature (Kantzioura et al., 2012). Numerous studies of particle (e.g. Sarigiannis et al., 2014; Yotova et al., 2016), chemical (e.g. Salapasidou et al., 2011; Samara et al., 2016), and radionuclide aerosols (Ioannidou et al., 2014), as well as bioaerosols, including pollen (e.g. Damialis et al., 2005; Voukantsis et al., 2010), fungal spores (e.g. Damialis and Gioulekas, 2006), microeukaryotes (Genitsaris et al., 2011a, 2014), and cyanobacteria (Genitsaris et al., 2011b), have been published. However, no study concerning the diversity, abundance and taxonomic composition of airborne bacteria, has been conducted in Thessaloniki.

2. Materials and methods

2.1. Air samplings

Air-dispersed bacteria were collected from the rooftop of the Biology Department (at a height of ca. 30 m) in the Aristotle University of Thessaloniki, located in the center of the city, using the SKC bio sampler (SKC Inc., PA, USA), via vacuum filtration with an average flow rate of 12.5 l of air min^{-1}. In total, 27 air samplings were conducted during the period June 2013–March 2014; 11 during June–July 2013, 7 during November–December 2013 and 9 in March 2014 (Table 1). For all samplings, data from 5 core meteorological parameters (air temperature, relative humidity, wind speed and direction, rainfall and total solar radiation) were measured by the Meteorological Department of Aristotle University of Thessaloniki and included in the analysis (Table 1). Wind direction was extrapolated for each sampling date and time, by "WindRose" graphical representations of wind direction from http://ready.arl.noaa.gov/READYcmet.php of the National Oceanic and Atmospheric Administration (NOAA), and expressed as degrees. Thus, North was expressed as 0° East as 90°, South as 180°, and West as 270°. Each air sampling lasted 3 h, which corresponded to a sample of 2.25 m^3 of air. For consistency, all samplings were conducted during daytime between 10 a.m. and 1 p.m. The airborne particles were trapped in a 100 mL medium, composed of distilled sterilized water (140 °C, 1.1bar for 15 min). Subsamples of 10 mL were filtered in Polycarbonate black filters (0.2 μm pore size, 25 mm diameter; Millipore, USA) and strained with 4′,6-diamidino-2-phenylindole (DAPI), at a final working concentration of 100 μg mL^{-1} for bacterial enumeration. In all samples, except one (30 March 2014 due to the presence of numerous inorganic particles), the DAPI staining was successful and we proceeded to the microscopic analysis. Furthermore, subsamples of 20 mL were filtered through 0.2 μm pore size membrane filters (Whatman, USA) and were immediately stored at −20 °C for molecular analysis. DNA extraction was attempted in all samples although subsequent molecular analysis was performed in 20 out of the 27 samples with the exception of those samples with low DNA yield (Table 1), possibly because of low quality of the extracted DNA.

2.2. Microscopic analysis

Bacterial cells were counted using a Nikon Eclipse TE 2000-S fluorescence microscopy under ultraviolet excitation at 1000× magnification, after the filters were fixed in sterile microscope slides. At least 100 bacterial cells were counted in each filter. Wet bacterial biomass was derived from biovolume calculation. For estimating bacterial biovolume the dimensions (length and width or diameter) of 30 cells of each bacterial phenotype in each filter were measured using a digital microscope camera (Nikon DS-L1). Next, the mean cell volumes for each phenotype were calculated after approximation to the nearest geometric formula. Finally, the mean cell volumes were multiplied by the total abundance of each phenotype, to calculate the bacterial biomass of each phenotype and the total bacterial biomass in each sample.

2.3. Molecular analysis

DNA from the filters was extracted and purified using the PowerSoil DNA isolation kit (MoBio Laboratories, USA), according to the manufacturer's protocol after slicing the filters with a sterile scalpel. The samples containing >0.5 ng μL^{-1} of DNA as measured by NanoDrop (Thermo Scientific, USA) were further processed by amplifying a domain (460–470 bp) of the V3-V4 hypervariable region of the 16S rRNA gene, using the two prokaryotic primers S-D-Bact-0341-b-s-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) (Klindworth et al., 2013).

PCR reactions and barcode amplicon sequencing process described by Dowd et al. (2008) was performed by the Mr. DNA Company (Shallowater, TX, USA; http://mrdnalab.com/). Briefly, an 8 bp tag sequencing specific to each sample, a 4 bp TCAG key and a 26 bp adapter for the FLX technology were added to the primers. A