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Bacterial community structure in atmospheric particulate matters of different sizes during the haze days in Xi'an, China

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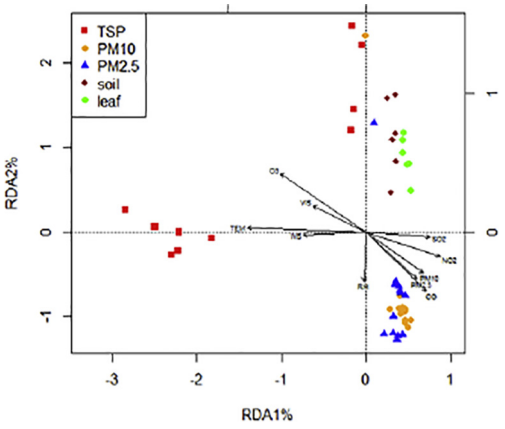
HIGHLIGHTS

- Bacteria community structures in $PM_{2.5}$, PM_{10} and TSP on hazy days were examined.
- High-throughput sequencing method was used for bacterial community profiles.
- Source tracking analysis was used to explore sources of airborne microbes.
- The bacterial abundance and diversity in different particle sizes are different.
- Haze pollution had no significant effects on bacterial community structures.

GRAPHICAL ABSTRACT

The distribution of bacterial communities with meteorological factors in different sample.



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ABSTRACT

Serious air pollution events have frequently occurred in China associated with the acceleration of urbanization and industrialization in recent years. Exposure to atmospheric particulate matter (PM) of high concentration can lead to adverse effects on human health. Airborne bacteria are important constituents of microbial aerosols and contain lots of pathogens. However, variations in bacterial community structure in atmospheric PM of different sizes ($PM_{2.5}$, PM_{10} and TSP) have not yet been explored. In this study, PM samples of different sizes were collected during the hazy days from Jul.2016 to Apr.2017 to determine bacterial diversity and community structure. Samples from soils and leaf surfaces were also collected to determine potential sources of bacterial aerosols. High-throughput sequencing technology was used generate bacterial community profiles, where we determined their diversity and abundances in the samples. Results showed that the dominant bacterial community structures in $PM_{2.5}$, PM_{10} and TSP were strongly similar. Compared with non-haze days, the relative abundances of most bacterial pathogens on the haze days did not increase. Meanwhile, temperature, O_3 and NO_2 had more significant effects on bacterial community than the other environmental factors. Source tracking analysis indicated that the airborne bacteria might be not from local environment. It may come from the entire city or other regions by long distance airflow transport. Results of this study improved our understanding of the influence of bioaerosols on human health and the potential sources of airborne microbes.

Abbreviations: TABs, total airborne microbes; $BioPM_{2.5}$, $BioPM_{10}$ and BioTSP, represent the concentration of airborne microbes in $PM_{2.5}$, PM_{10} and TSP; VIS, visibility; TEM, temperature.

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1. Introduction

Bioaerosols, including bacteria, fungi, viruses, pollen, spores, animal and plant debris, can contribute to as much as 25% of the atmospheric aerosols (Ariya and Amyot, 2004; Jaenicke, 2005). These bioaerosols play key roles in the Earth's system through their interactions with the atmosphere, which in turn influence the climate and affect public health. Specifically, microbes in the atmosphere enter the human body through the damaged skin, mucous membrane, digestive and respiratory tracts, posing threats to human health and causing a variety of diseases, including infections, acute toxic effects, allergies and even cancers (Biermann et al., 2013; Li et al., 2015; Walser et al., 2015). These resulted in increasing number of studies looking at characteristics of bioaerosols in the ambient air over the past decade.

Many quantitative and qualitative studies have been done on culturable microbial aerosols, though culture-based bioaerosols only accounted for 0.1%–10% of the total microbial biomass (Concepts and Macher, 2014; Haas et al., 2013; Li et al., 2015; Nasir and Colbeck, 2010). This limitation in methods resulted in some important information about pathogenic bacteria and allergens being neglected since non-cultivable species are not detected. In contrast, molecular-based high-throughput sequencing technology has been shown to be an effective method to acquire another layer of information on bacterial diversity and community structures. The advent of this technology provides an opportunity to fully understand both culturable and nonculturable airborne microbes.

Bacterial cell size typically ranges from 0.5 to 2.0 μm, but bacterial cells could also form clumps or attach to other larger particles. As a result, bioaerosols generally ranges from 0.3 to 100 μm in diameter (Lighthart and Mohr, 1994). However, most studies on bacterial community structures in PM have only focused on finer particles ($PM_{2.5}$), and information on $PM_{2.5}$, PM_{10} and TSP remains little. It is then essential to investigate bacterial ecology in the different sizes of particulate matter.

Recently, serious air pollution events or hazy days have frequently occurred in China. High PM concentrations have been reported during these haze days (Quan et al., 2014; Sun et al., 2006; Yang et al., 2012). Several studies further indicated that the concentrations of total airborne microbes (TABs) or viable bioaerosols on hazy and foggy days are significantly higher than non-hazy days (Li et al., 2015; Dong et al., 2016; Wei et al., 2016; Li et al., 2017; Xie et al., 2017). Trends in PM also showed shifting peak of concentration to fine size range during haze days (Li et al., 2015). Some studies observed that bacterial communities were very similar between autumn and winter, but the dominant species differed. These studies however were just aimed at the bacterial community structures in single particulate size (Cao et al., 2014; Du et al., 2017; Gou et al., 2016). Little is still known about the differences in bacterial community and diversity in bioaerosols during the same sampling period between haze and non-haze days, especially in different sizes of particulate matter.

In addition, the environmental factors such as relative humidity, temperature, wind direction, wind speed and gas pollutants (O_3, SO_2, NO_2, CO, etc.) had different degrees of influences on bacterial community and diversity (Jones and Harrison, 2004; Zhen et al., 2017). Xu et al. (2016) found O_3 and SO_2 to critically contribute to the variation in bacterial community in Shandong. Zhen et al. (2017) reported that bacterial abundances in spring and autumn were higher than those in winter, which could be attributed to temperature differences between seasons. These show that, the bacterial community and diversity are strongly influenced by different environment conditions.

Most studies have also been focusing on the potential sources of airborne bacteria, but were not able to reach a uniform conclusion about the sources (Bowers et al., 2013; Bowers et al., 2011; Cao et al., 2014). For example, Cao et al. (2014) and Bowers et al. (2013) pointed that bioaerosols originated from any environmental reservoir for microorganism, such as soil, leaves and animals. On the contrary, some other researchers found that dusts directly affected airborne bacterial communities by air mass transport (Jeon et al., 2011; Maki et al., 2015). These studies indicate that pinpointing bacterial sources is more complicated than previously thought. Many factors need to be accounted for, such as natural, and anthropogenic sources, weather conditions (rain, dust storms and haze pollution) and the typical surrounding environment (farms, ranches, factory, etc.) (Bowers et al., 2013; Huffman et al., 2010).

This study collected bioaerosol samples in Xi'an City, the largest semi-arid inland city in China. Samples collected were assayed by fluorescent microscopic method and used for high-throughput sequencing to generate bacterial community profiles. We aim to improve our understanding of bacteria diversity and community structures in various sizes of particulate matter ($PM_{2.5}$, PM_{10} and TSP) on heavily hazy days. Furthermore, backward trajectory analysis was performed to reveal the possible sources of airborne microbes. Results of this study provide valuable data for hazard evaluation of bioaerosols on human health during the haze days, and also improves our understanding of the potential sources of airborne bacteria.

2. Materials and methods

2.1. Sampling sites and samples collection

Airborne PM samples were collected in Chang'an University from October 2016 to January 2017 in Xi'an, China (34.23° N, 108.96° E, and 424 m above sea level). The sampling sites are situated between the 2nd and 3rd ring roads in Xi'an City. The sampling sites are 400 m away from the nearest major roads. Surrounding the sites are trees, greenbelts, residential and school buildings with no identified potential industrial pollution sources. An air sampler (ZR-3930, Qingdao, China) was used to collect TSP、PM_{10} and $PM_{2.5}$ (see Supplementary Fig. S1) onto 47 mm (diameter) sterilized polycarbonate membranes (Whatman, UK). All experimental vessels underwent high-temperature sterilization at 121 °C in a pressure steam sterilizer (LDZX-40BI, Shang'hai, China) for 20 min.

The environmental indices ($PM_{2.5}$, PM_{10}, SO_2, NO_2, CO, O_3, and AQI) were obtained from a portable automatic meteorological station (PH-1, Wuhan, China), which was installed near the sample sites. The air quality index (AQI) was generally applied to assess the air quality by China Meteorological Administration (CMA). It derived concentration of six major air pollutants, namely $PM_{2.5}$, PM_{10}, SO_2, NO_2, CO and O_3. AQI ($PM_{2.5}$) was assigned to the level of $PM_{2.5}$ in this study. When the value was >100, which is the limit for Chinese air quality standards Grade II, the environmental condition was categorized as hazy pollution. Based on these criteria, 7 hazy days and 5 non-hazy days were identified from the total of 12 sampling days included in this study. The environmental indices during this sampling period were presented in Table 1.

2.2. Fluorescent microscopic analysis of bioaerosols

The sampler was used with a flow rate of 16.7 L/min, and a duration of 15 min was set for each fluorescent sample of $PM_{2.5}$, PM_{10} and TSP. After sampling, the polycarbonate membranes with bioaerosols were immediately transferred to 250 mL Erlenmeyer flasks containing 30 mL of phosphate buffer and 3 mL of Tween-80 solution, followed by shaking at 120 rad/min for 20 min. Next, the samples were filtered through a black Nuclepore Track-Etch membrane (Whatman, UK) and stained with 10 μg/mL DAPI (4′,6-diamidino-2-phenylindole) for 20 min in the dark. Then, DAPI-stained cells from 10 randomly selected ocular fields were counted using a fluorescent microscope (Shanghai Cewei Photoelectric Technology Co., Ltd, China) equipped with a UV light. The microscopic view of fluorescent stained samples is presented in Fig. S2. The standard deviation of the selected 10 microscopic views counted in every sample was calculated, averaging 2.98 from