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Effect of air pollution on the total bacteria and pathogenic bacteria in different sizes of particulate matter☆

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ABSTRACT

In recent years, air pollution events have occurred frequently in China during the winter. Most studies have focused on the physical and chemical composition of polluted air. Some studies have examined the bacterial bioaerosols both indoors and outdoors. But few studies have focused on the relationship between air pollution and bacteria, especially pathogenic bacteria. Airborne PM samples with different diameters and different air quality index values were collected in Hangzhou, China from December 2014 to January 2015. High-throughput sequencing of 16S rRNA was used to categorize the airborne bacteria. Based on the NCBI database, the "Human Pathogen Database" was established, which is related to human health. Among all the PM samples, the diversity and concentration of total bacteria were lowest in the moderately or heavily polluted air. However, in the PM2.5 and PM10 samples, the relative abundances of pathogenic bacteria were highest in the heavily and moderately polluted air respectively. Considering the PM samples with different particle sizes, the diversities of total bacteria and the proportion of pathogenic bacteria in the PM10 samples were different from those in the PM2.5 and TSP samples. The composition of PM samples with different sizes range may be responsible for the variances. The relative humidity, carbon monoxide and ozone concentrations were the main factors, which affected the diversity of total bacteria and the proportion of pathogenic bacteria. Among the different environmental samples, the compositions of the total bacteria were very similar in all the airborne PM samples, but different from those in the water, surface soil, and ground dust samples. Which may be attributed to that the long-distance transport of the airflow may influence the composition of the airborne bacteria. This study of the pathogenic bacteria in airborne PM samples can provide a reference for environmental and public health researchers.

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1. Introduction

Outdoor air pollution causes 3.3 million premature deaths per year worldwide, foremost in Asia (Lelieveld et al., 2015). Among the various factors of polluted air, particulate matter (PM) plays an important role in posing a risk to human health (Chang et al., 2009). Total suspended particulate (TSP) attached on the skin and clog pores. PM10 (particulate matter with aerodynamic diameter $\leq$10 μm) inhaled via the upper respiratory tract and induce cytotoxicity and inflammation (Happo et al., 2014). PM2.5 (particulate matter with aerodynamic diameter $\leq$2.5 μm) can easily penetrate to the lungs and bloodstream tissues, causing respiratory and vascular diseases (Lelieveld et al., 2015).

In addition to the physical and chemical components of airborne PM, the bacteriological component also exist in the PM (Darwin, 1846). Some researchers have reported that bacteria can influence public health and ecological systems. The effects of the long-distance airborne bacterial transmission can extend to entire ecosystems; bacteria in dust from the Sahara Desert affected the Spanish alpine lake (Barberán et al., 2014) and Caribbean coral reefs (Prospero et al., 2005). Pathogenic bacteria in air can induce several human diseases (Balloy and Chignard, 2009; Cerdeño-Tárragaet al.,

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2003). *Legionella pneumophila*can cause severe pneumonia especially Legionnaires' disease. *Legionella* can be transmitted and inhaled with the contaminated aerosols (Allegra et al., 2016; Daumas et al., 2012; Yamaguchi et al., 2017). *Mycobacterium tuberculosis*is responsible for tuberculosis, which can easily spread in the air (Eames et al., 2009). *Staphylococcus aureus* causes abscesses (Masalha et al., 2001) and may attach to skin in an aerosol form (Eames et al., 2009). *Clostridium difficile* is responsible for inflammation and is thought to be transmitted in the air (Roberts et al., 2008).

For the outdoor airborne bacteria, some researchers focused on their concentration, diversity, sources and environmental impact factors (Alghamdi et al., 2014; Barberán et al., 2015; Gonzalez et al., 2000; Hara and Zhang, 2012). On dusty days the concentrations of total bacterial cell were higher than those on non-dusty days (Hara and Zhang, 2012). On hazy days, the concentrations of detected bioaerosol were more than 6-fold higher than those on sunny days (Wei et al., 2016). Considering PM of different diameters, the diversity of total bacteria was higher in PM10 sample than in PM2.5 sample (Alghamdi et al., 2014). Various potential sources, such as water, soil, plants, animals, and human activities may affect the distribution of airborne bacteria (Gonzalez et al., 2000; Gangamma et al., 2011). Overall, the bacteria in outdoor air samples are predominantly derived from soil and plants (Barberán et al., 2015; Gandolfi et al., 2015). Factors such as the soil pH, mean annual precipitation, net primary productivity, and mean annual temperature are responsible for the variability observed in bacterial composition (Barberán et al., 2015).

Although several allergens and pathogens have been observed in previous reports (Cao et al., 2014; Woo et al., 2013), it is not clear about the properties of the pathogenic bacteria in the air. In this study, we have collected airborne PM samples with various particle sizes and different AQI in Hangzhou (Table S1) from December 2014 to January 2015. In addition, a pathogen library named the "Human Pathogen Database" was established. We hypothesized that compositions of total bacteria and pathogenic bacteria were influenced by the polluted air, PM sizes and environmental factors. Based on this hypothesis, the diversity of the total airborne bacteria and the relative abundance of pathogenic bacteria were studied in different PM sizes and at different air quality levels. In addition, we also hypothesized that the potential source environment would affect the structure of the total bacteria community. To test the hypothesis, the adjacent surface soil, ground dust, and water samples were collected as the potential source environmental samples.

2. Materials and methods

2.1. Sample collection

Airborne PM samples were collected from the rooftop of a building in Hangzhou, China (30° 16′ N, 120° 07′ E, ~10 m above the ground). The sampling site is located approximately 10 m away from the nearby rivulet, and is approximately 15 m in distance from Zijinhua Road. PM was collected using a high-volume air sampler (Laoshan Application, Qingdao, China) with a flow of 1.05 m^3 min^{-1}. The sampling filter membrane (Whatman, UK) was an ordinary rectangular glass fiber membrane with an effective size of 230 × 180 mm, which underwent high-temperature sterilization at 100 °C in a muffle furnace for 12 h. From December 2014 to January 2015, the PM samples were collected on different days by the same sampler. According to the air quality levels and particulate sizes, 11 airborne PM samples were selected. Every air sample was collected for 12 h every day from 8 a.m. to 8 p.m. After sampling the membranes were stored at −20 °C.

Hourly air pollutants data were collected from local air quality monitoring station (Table S1), including PM2.5, PM10, carbon monoxide (CO), nitrogen dioxide (NO_2), ozone (O_3) and sulfur dioxide (SO_2) concentrations. In addition, the corresponding hourly meteorological data were also collected from the local meteorological monitoring station (Table S1), including air temperature (T), relative humidity (RH), and wind scale (WS). According to the Technical Regulation on Ambient Air Quality Index (HJ 663−2012, China), the air quality were divided into six indexes, which were described as good, moderate, lightly polluted, moderately polluted, heavily polluted and severely polluted air. Except severely polluted air quality, the samples were collected with five different air quality indexes. The number of sample's description represented the corresponding air quality index. The PM2.5, PM10 and TSP were described as A, B and C, respectively (Table S1).

Samples with different PM sizes and various air quality levels were categorized to analyze the total and pathogenic bacteria community compositions (Table S1). Four PM2.5 samples (A2, A3, A4, and A5) were categorized from moderate to heavily polluted air quality levels. Four PM10 samples (B1, B2, B3, and B4) were collected from good to moderately polluted air. Three TSP samples (C2, C3, and C4) were selected, ranging from moderate to moderately polluted air quality levels. At the same level of air quality, different PM sizes may affect the total bacterial and pathogenic bacteria community structures. Furthermore, two water samples (E1 and E2), two surface soil samples (E3 and E4) and two ground dust samples (E5 and E6) were collected as surrounding environmental samples within 10 m of the airborne PM sampling site.

2.2. DNA extraction and PCR amplification

DNA was extracted from the airborne PM samples using a Power Soil DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA). Firstly, the filter was divided into 8 equal parts. Every part contained both the center and the edges of a filter. Secondly, 1/8th of a filter with PM2.5, PM10 or TSP were cut into pieces and loaded into the bead tubes. Then, the tubes were heated to 65 °C for 10 min, followed by shaking for 20 min. The remaining steps were performed according to the manufacturer's instructions. The soil and dust samples were extracted directly using this kit. The water samples were extracted by Power Water DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA). The nucleic acid concentration sensor NanoDrop 2000 (Thermo Fisher Scientific, Merinton, USA) was used to check DNA quality. Additionally, the DNA concentration was detected by Qubit Fluorometer (Thermo Fisher Scientific, Merinton, USA). To quantify the total bacteria, quantitative PCR (qPCR) based on SYBR Green was used. Forward primer 515F and reverse primer 806R of total bacteria were used. The plasmid DNA was 10-fold diluted to construct the standard curve. Then, according to the threshold cycle values of the standard curve, the concentrations of bacterial cells were calculated. The detailed qPCR protocol was described in previously reported work (Ren et al., 2015). For sequence analyses, 16S rRNA genes were amplified using the primer pair 515-F (5′-GT GCC AGC MGC CGC GG-3′) and 907-R (5′-AGA CAT GGT GCC AGC MGC CGC GG-3′). A AxyPrep DNA Gel Extraction Kit (Axygen, Silicon Valley, USA) was used to purify the PCR amplification products. All purified amplified DNA samples were stored at −20 °C for downstream analysis (Hu et al., 2011). High-throughput sequencing of 16S rRNA was conducted using the Ion Torrent sequencing platform. Blank filters and DNase/RNase-Free water were analyzed with the same procedure as the negative controls. After extraction and amplification, the DNA concentrations of the two kinds of negative controls were below the detection limit.

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