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# Bacterial characterization in ambient submicron particles during severe haze episodes at Ji'nan, China

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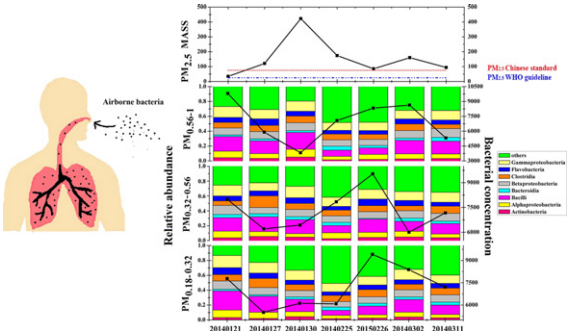
## HIGHLIGHTS

- High bacterial concentration and diverse bacterial community in submicron particles during haze episodes were observed.
- The bacterial community varied significantly via different size fractions.
- Source track analysis showed that the ambient bacteria mainly originated from soils, leaf surfaces, and feces.

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## ABSTRACT

In January 2014, severe haze episodes which sweep across Chinese cities have attracted public concern and interest at home and abroad. In addition to the physicochemical properties of air pollutants, bacteria are thought to be responsible for the spread of respiratory diseases and various allergies. We attempted the bacterial characterization of submicron particles ($PM_{0.18–0.32}$, $PM_{0.32–0.56}$, and $PM_{0.56–1}$) under severe haze episodes using high-throughput sequencing and real-time quantitative PCR detecting system based on 21 samples collected from January to March 2014 at Ji'nan, China. The high bacterial concentration in $PM_{0.32–0.56}$ (7314 cells $m^{-3}$), $PM_{0.18–0.32}$ (7212 cells $m^{-3}$), and $PM_{0.56–1}$ (6982 cells $m^{-3}$) showed significant negative correlations with $SO_2$, $NO_2$, and $O_3$. Under sufficient sequencing depth, 37 phyla, 71 classes, 137 orders, 236 families, and 378 genera were classified, and the bacterial community structure varied significantly in different size fractions. For example, Holophagaceae (Acidobacteria) in $PM_{0.32–0.56}$ showed 6-fold higher abundance than that in $PM_{0.18–0.32}$. Moreover, functional categories and bacterial species (*Lactococcus piscium*, *Pseudomonas fragi*, *Streptococcus agalactiae*, and *Pseudomonas cichorii*) that may potentially be responsible for infections and allergies were also discovered. Source track analysis showed that the ambient bacteria mainly originated from soils, leaf surfaces, and feces. Our results highlighted the importance of airborne microbial communities by understanding the concentration, structure, ecological and health effects, especially those in submicron particles during haze episodes.



## 1. Introduction

In the last few decades, the rapid economic growth and energy consumption, along with the lack of measures for protecting atmospheric environments, has resulted in continuous haze episodes in China (Yang et al., 2015; Li and Zhang, 2014). In severe haze episodes, the

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daily average $PM_{2.5}$ mass concentration of Jing-Jin-Ji regions (Wang et al., 2013; Han et al., 2016) largely exceeded 25 μg $m^{-3}$, which is specified as the limit of the World Health Organization(WHO) $PM_{2.5}$ health guideline, by about 20-fold. Exposure to such high concentrations of airborne particles leads to high morbidity and mortality due to infectious diseases such as cardiovascular diseases, respiratory infections, and lung cancer (Bowers et al., 2013; Esposito et al., 2012). Based on the definition of haze by the State Standard of the People's Republic of China (QX/T 113-2010), haze is defined as a complex air pollution process include the following conditions: (1) visibility <10 km and relative humidity lower than 80%, and (2) the $PM_{2.5}$ mass concentration higher than 75 μg $m^{-3}$ (Leng et al., 2013; Kong et al., 2014; Jansen et al., 2014; China Meteorological Administration, 2010). In January of 2013 and 2014, severe haze episodes were reported in Beijing (Wei et al., 2016), Hebei (Wang et al., 2013), Nanjing (Kong et al., 2015), and Ji'nan (L.W. Wang et al., 2015) which caused great economic losses and public panic across China. Ji'nan is the capital of Shandong Province and covers an area of 8177 $km^2$. It is surrounded by hills on three sides which may exacerbate the accumulation of airborne pollutants including atmospheric particles, sulfur dioxide, nitrogen oxide, trace gases, and volatile organic compounds (Liu et al., 2015; Zhang et al., 2014; Li et al., 2011). Majority of existing studies focussed on the bacteria which occupied about 80.8% and 86.1% of the total microbes (Cao et al., 2014) in $PM_{2.5}$ and $PM_{10}$. Limited studies investigated bacteria in submicron particles (Gou et al., 2016), which can easily penetrate lungs or even the blood stream (Janssen et al., 2011; Visser et al., 2015; Gao et al., 2015b). Hence it is essential to study the bacterial characteristics of such aforementioned submicron particles in atmosphere.

The near-surface and upper troposphere contain thousands to millions of bacterial cells per cubic meter (Bowers et al., 2012). Active bacteria can serve as a medium for the spread of allergens and pathogens in a crowd (Creamean et al., 2013; Husman, 1996). There are also increasing evidence indicating that bacteria can act as cloud condensation nuclei, absorbing or reflecting sunlight, or even participating in N-cycling and C-cycling in the ecosystem (Bauer et al., 2003). So far, many investigations on the active bacterial concentration and bacterial community in airborne particles have been conducted (Bertolini et al., 2013; Hospodsky et al., 2015; Prussin et al., 2015). The airborne bacterial concentration in the near-surface ranged from $10^4$ to $10^6$ cells $m^{-3}$ (Bowers et al., 2012; Haas et al., 2013; Murata and Zhang, 2014; Goudarzi et al., 2014; Murata and Zhang, 2016). Bowers et al. (2013) reported detailed information on the airborne microbial community and sources in $PM_{2.5}$ and $PM_{2.5-10}$ and found that the bacterial richness and communities structures showed a significant distinction across these two size fractions. In China, Cao et al. (2014) described the microbial communities of $PM_{2.5}$ and $PM_{10}$ using metagenomics during a serious smog event and found that bacteria were the dominant one which was mostly terrestrial-related. Wei et al. (2016) investigated the concentration and size distribution of bioaerosols during haze and sunny days in Beijing. Compared to the sunny day, the fluorescent particle concentrations increased during the haze episodes and decreased with the dissipation of haze occurrences in 3–5 days. Furthermore no obvious difference in the airborne bacterial abundance and community structure were observed between haze and sunny days. Although these studies have illustrated the concentration and community compositions of cultured or uncultured bacteria in atmospheric fine particles, studies on bacterial characterizations in submicron particles are rare, especially during severe haze episodes.

Herein, we first characterized severe haze episodes to reveal the nutrients in submicron particles from January to March 2014 in Ji'nan. The bacterial concentration and community structure of different particle size fractions was analyzed subsequently. Third, we performed functional analysis of the bacteria in the submicron particles to assess their potential to cause risk to human health. Our study draws a framework of bacterial community in Jinan's submicron particles during haze episodes and emphasizes the health risks of long-term exposure to high concentrations bacteria.

## 2. Materials and methods

### 2.1. Aerosol collection

Aerosol samples were collected from the rooftop of the Lizong building in the central campus of Shandong University located in Ji'nan (36°40′N, 117°3′E). The Lizong building is a six-floored teaching building where classes are conducted from 08:00 to 17:00 from Monday to Friday. To avoid the interference from local anthropogenic emissions on the ground, a Micro-Orifice Uniform Deposit Impactor (MOUDI) and on-line monitoring instruments such as $SO_2$ analyzer (Model 43C, Thermo, USA), NOx analyzer (Model 42C, Thermo, USA), $O_3$ analyzer (Model 49C, Thermo, USA), and CO analyzer (Model 300E, Teledyne API, USA) were placed in the rooftop of Lizong building about 20 m from the ground. We sterilized the quartz membrane by baking in a Muffle furnace at 500 °C for 6 h before sampling. After the filter cooled, it was packaged into sterilized aluminum foil and stored in a sealed bag. Before sampling, the inside surfaces of the MOUDI were kept sterile and 75% ethanol was used to sterilize the impactor. Seven sets of aerosol samples were obtained on the 47-mm quartz membrane of the MOUDI for 23 h (9:00 am to 8:00 am next day) at a flow rate of 30 lpm during Jan. 20, 2014 to Mar. 31, 2014; these samples were stored at −80 °C until analysis. Each set contained nine samples in nine size-resolved ranges as follows: stage1, ≥18 μm; stage2, 10–18 μm; stage3, 5.6–10 μm; stage4, 3.2–5.6 μm; stage5, 1.8–3.2 μm; stage6, 1.0–1.8 μm; stage7, 0.56–1.0 μm; stage8, 0.32–0.56 μm; and stage9, 0.18–0.32 μm. The $PM_{1.0}$ can easily penetrate thoracic and pulmonary airways and plays an important role in haze formation and visibility degradation (Shi et al., 2014). Meanwhile the fact that specific surface area of $PM_{1.0}$ is greater than $PM_{2.5}$ provides evidence that $PM_{1.0}$ containing more health risks. Therefore we used the stage7, stage8, and stage9 samples for the following experiments. An automatic meteorological station (JZYG, PC-4) was employed to measure meteorological factors (wind direction, wind speed, humidity, and temperature) in real time. Meanwhile, a Synchronized Hybrid Ambient Real-Time Particulate monitor (SHARP, Model 5030, Thermo Fisher Scientific, USA) and a Monitor for AeRosols and GAses analyzer (MARGA, ADI20801, Applikon-ECN, Netherlands) were used to analyze the hourly average mass concentration of $PM_{2.5}$, water-soluble ions, and trace gases as described previously. Based on the definition of haze, seven days including six haze days (January 27, 2014; January 30, 2014; February 25, 2014; February 26, 2014; March 2, 2014; and March 11, 2014) and one clear day (January 21, 2014) were selected. Details about sampling time and the chemical characteristics including $PM_{2.5}$, trace gases ($SO_2$, $NO_2$, and $NH_3$), water soluble inorganic ions ($NH_4^+$, $SO_4^{2-}$, $NH_4^+$, $K^+$, $Cl^-$, and $Na^+$), and meteorological factors (wind direction and wind speed) of sampling time are summarized in Fig. 1.

### 2.2. DNA extraction and PCR amplification

DNA was extracted from the quartz membrane fragments (cut into 1.1 $cm^2$ filter area) using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. DNA concentration was determined using NanoDrop 2000 (Thermo, Wilmington, Delaware, USA). Extracted DNA samples were stored at −80 °C until further analysis. The V3-V4 region of 16S rRNA was amplified using a bacterial universal PCR primer set 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 907R (5′-CCYCAAT TCMTTTRAGTTT-3′) (Gallagher et al., 2013). PCR amplification was performed on an ABI GeneAmp® PCR system 9700 (Applied Biosystems, 101 Foster City, CA) using a 20 μL reaction mixture contained 4 μL 5 × FastPfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL 5 μM forward primer, 0.8 μL 5 μM reverse primer, 0.4 μL Fastfu polymerase, 10 ng template