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Investigation of diverse bacteria in cloud water at Mt. Tai, China

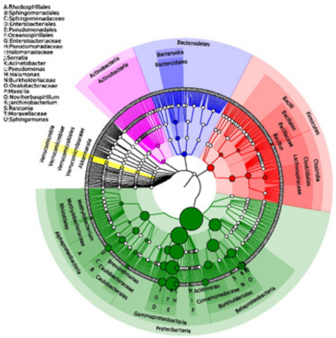
Caihong Xu [a], Min Wei [a], Jianmin Chen [a,b,*], Xiao Sui [a], Chao Zhu [a], Jiarong Li [a], Lulu Zheng [b], Guodong Sui [b], Weijun Li [a], Wenxing Wang [a], Qingzhu Zhang [a], Abdelwahid Mellouki [b,c]

[a] *Environment Research Institute, School of Environmental Science and Engineering, Shandong University, Ji'nan 250100, China*
[b] *Shanghai Key Laboratory of Atmospheric Particle Pollution and Prevention (LAP), Fudan Tyndall Centre, Department of Environmental Science & Engineering, Fudan University, Shanghai 200433, China*
[c] *Institut de Combustion, Aérothermique, Réactivité et Environnement, CNRS, 45071 Orléans cedex 02, France*

HIGHLIGHTS

- Bacterial community in cloud water of Asian area was studied for the first time.
- High throughput sequencing revealed diverse gram-negative bacteria mainly inhabiting in leaf-surface and cold environments.
- Bacteria involved in the cloud condensation nuclei and ice nucleation process were observed.
- SO_2 and O_3 distinctly contributed to the variations of species-environment relationship in different samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacteria are abundant in atmospheric water phase with the potential to influence atmospheric processes and human health, yet relatively little information is known about the bacterial characteristics at high altitudes. Here we investigated the bacterial community by high throughput sequencing in 24 cloud water samples collected from September 26 to October 31, at the summit of Mt. Tai (36°15′ N, 117°06′ E, 1534 m a.s.l) in China. Diverse bacterial population were identified and the gram-negative bacteria contributed the majority of total bacteria including Proteobacteria (81.6%) and Bacteroidetes (3.9%), followed by gram-positive bacteria Firmicutes (7.1%) and Actinobacteria (2.3%). These gram-negative taxa mainly inhabited in leaf-surface and cold environments. Meanwhile bacteria involved in the cloud condensation nuclei and ice nuclei formation were observed such as *Sphingomonas* (6.7%), *Pseudomonas* (4.1%), and *Bacillus* (1.1%). In addition, *Sphingmonas* was more active than that in daytime and participated in the cloud chemistry process. Meanwhile O_3 and SO_2 critically contributed to the variation of bacterial community. It is the first report on the bacterial community structure of cloud water over Asian area. Our results can serve as an important reference for environmental scientists, and biologists.

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* Corresponding author at: Environment Research Institute, School of Environmental Science and Engineering, Shandong University, Ji'nan 250100, China.
E-mail addresses: jmchen@sdu.edu.cn, jmchen@fudan.edu.cn (J. Chen).

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1. Introduction

Airborne bacteria have gained growing attentions from scholars, mainly focusing on their potential roles in atmospheric chemistry and nucleation process such as biotransformation of organic matter, carbon cycling, photochemical reactions, and cloud formation (Amato et al., 2007a; Bowers et al., 2009; Delort et al., 2010; Despres et al., 2012; Šantl-Temkiv et al., 2015; Smets et al., 2016). These microbes also provide a medium for the spread of diseases through potential pathogens and allergens (Bowers et al., 2012; Cao et al., 2014). The previous findings have reported that these bacteria at high altitudes are metabolic active and reproductive even under a hostile super-cooled environment (Smets et al., 2016). Increasing evidence stated that the active bacteria isolated from different cloud type (continental, marine, and anthropogenic cloud) can link to the photochemistry and biological reactions (Delort et al., 2010; Vaïtilingom et al., 2012). While the bacterial characteristics obtained from orographic clouds have not been fully studied.

Orographic clouds are generated by the forced lift of air mass under a special topographic feature, in particular mountain regions. The air mass can quickly cool down adiabatically with the increase of altitudes, and then raise the relative humidity up to 100%, a fact that is likely to influence the bacterial community (Kourtev et al., 2011). So far the research at the high altitudes were restricted, mainly focused on the bacterial ice nucleation activity, concentration and functions on the nucleation process (Matthias-Maser et al., 2000; Birgit et al., 2001; Marinoni et al., 2004; Vaïtilingom et al., 2012; Joly et al., 2013; Amato et al., 2015). Researches on the total bacterial community were rare due to the limited measurement technique. Vaïtilingom et al. (2012) investigated the microbial population in cloud water via cultured method at the puy de Dôme Mountain (about 1465 m), France. Yet, this method restricted on the cultured bacteria which contributed to <1% of the total bacteria (Amato et al., 2007a; Hill et al., 2007). With the advances in high-throughput sequencing, many researchers employed this technology to characterize the taxonomic identification of airborne bacteria at phylum, class, order, family, genus, and species levels. Bowers et al. (2009) presented the dominant Proteobacteria and Bacteroidetes which occupied about 93% of total bacteria by tagged pyrosequencing, and evaluated their potentials to act as ice nuclei at Mt. Werner (about 3200 m), USA. Afterward at the same sampling site, they found the temperature affected the seasonal variation of airborne community dramatically (Bowers et al., 2012). DeLeon-Rodriguez et al. (2013) revealed an important impact of hurricanes on the bacterial community in the middle to upper troposphere across the United States. Maki et al. (2015) reported the metrological influence on the bioaerosol collected at three different altitudes (10 m, 1000 m, and 3000 m) in downtown atmosphere over the Noto Peninsula, Japan. However, the relevant researches were mostly conducted in America and Asia based on the aerosol samples, limited eyes concentrated on the bacterial population in cloud water across Asian region. Hence, it is essential to advance the knowledge, especially in the fast developing countries. During 2013, 2014 and 2015, Northern China experienced serious air pollution associated with the inadequate use of energy in the transport, domestic, and industrial sectors. North China Plain is the region with the worst air pollution in China, including Beijing, Tianjin, Shijiazhuang, Jinan, and Qingdao. Mt. Tai (36°15′ N, 117°06′ E, 1534 m a.s.l), locates on the summit of North China Plain, covers a total area of approximately 426 km^2, faces the east sea and back on the yellow river to the west. It is frequently attacked by cloud events, especially in March, April, June, July, October, and November (Guo et al., 2012; Liu et al., 2012; Wang et al., 2011). The special terrain is conducive to the formation of orographic clouds. Across this area, researchers have described the physicochemical properties of cloud water, e.g. pH, conductivity, water soluble inorganic ions, sulfur (IV), total organic carbon, PAHs, heavy metal elements and organic acids (Li et al., 2011a; Li et al., 2011b; Li et al., 2013; Ervens, 2015; Li et al., 2016), and did not pay attentions on the airborne bacterial characteristics.

Herein, we used high-throughput sequencing to characterize the bacterial communities in cloud water collected at Mt. Tai, China, with environmental factors ($PM_{2.5}$, SO_2, O_3, CO, and NO_2) monitored in real time. The unique dataset fulfill the gap of the comprehensive analysis of bacterial communities at a high altitude site across the Asian area and allow us to address the following hypothesis: what characteristics the bacterial community in cloud water at high altitude exhibited? Does the bacterial diversity and community structure varied between daytime and nighttime? Does the bacterial community structure changed across different environmental factors?

2. Material and methods

2.1. Cloud water collection and environmental factors

Cloud water is formed by water condensation of atmospheric particles under supersaturation environment (Martinsson et al., 1999). We determined the cloud events based on the duration. The cloud process lasted >1 h in a day was considered as one cloud event. For each event, continuous samples were collected once volumes got enough of 100 mL. As shown in Table 1, Mt. Tai experienced 10 cloud events, and 24 samples were collected from September 26 to October 31, 2014. The cloud water were sampled on a bank of Teflon strands at 24.3 m^3/min by a fan situated at the rear by Caltech Active Strand Cloud water Collector Version 2 (CASCC2) (Collett et al., 2001; Wang et al., 2014; Modini et al., 2015). Collected samples flowed from the Teflon strands, through Teflon tube into a polyethylene bottle finally. The theoretical 50% cut-off size was equivalently drop diameter of 3.5 μm. To avoid the artificial contamination, the Teflon strands and tube were kept in sterile criteria and the polyethylene bottles were pretreated with 75% ethanol before sampling. The pH and conductivity of the samples were measured immediately using a pH/conductivity analyzer (pH/COND/TEMP METER, 6350). An automatic meteorological station (JZYG, PC-4) and Synchronized Hybrid Ambient Real-Time Particulate monitor (SHARP, Model 5030, Thermo Fisher Scientific, USA) were used to measure the environmental temperature and $PM_{2.5}$ mass concentration in real time. Trace gases SO_2, NO_2, and O_3 in atmosphere were monitored by Model 42C, Model 43C, and Model 49C (Thermo Scientific, USA). CO was monitored by an API 300E analyzer (Teledyne, USA). The Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model was employed to create the twenty-four hours backward trajectories for air mass transport pathway arriving at 1534 m above ground level. The detail information about the sampling time and environmental factors were shown in Table 1. In order to identify the day/night variation of bacterial community, we specified daytime sampling from 7 a.m. to 7 p.m. Beijing time and nighttime sampling from 7 p.m. to the next day 7 a.m. Beijing time. There were twelve samples sampled from daytime and nighttime.

2.2. DNA extraction and PCR amplification

Firstly, each sample was centrifuged for 3 min at 3000 rpm, then discarded the supernatants and extracted the pellets. Genomic DNA was extracted by a MP Biomedicals FastDNA Spin Kit for soil according to the manufacturer's directions. Afterwards DNA concentration was analyzed by NanoDrop Spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). The ultrapure water and a MP Biomedicals FastDNA Spin Kit blank served as negative controls to indicate any microbial contamination in the sampling and extraction kit. The V3-V4 hypervariable regions were amplified with the PCR primer pairs 341f (5′-CCTACGGGNGGCWGCAG) with adaptor B and 805r (5′-GACTACHVGGGTATCTAATCC) with adaptor A (Ye et al., 2013; Herlemann et al., 2011), and the barcode nucleotide sequences was equipped between PCR primer and adaptor. The PCR amplification was performed on an ABI GeneAmp® PCR system 9700 (Applied Biosystems, 101 Foster City, CA) using a 20 μL-mixture containing 4 μL