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Cell concentration, viability and culture composition of airborne bacteria during a dust event in Beijing

Hongli Yuan[1,*], *Daizhou Zhang*[2,*], *Yanning Shi*[1], *Baozhen Li*[1], *Jinshui Yang*[1], *Xuejian Yu*[1], *Nan Chen*[1], *Makiko Kakikawa*[3]

1. *State Key Laboratory of Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing 100193, China*
2. *Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto 862-8502, Japan*
3. *Institute of Nature and Environmental Technology, Kanazawa University, Kanazawa 920-1192, Japan*

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ABSTRACT

Airborne bacteria were measured when a dust storm passed Beijing in spring 2012 with a focus on cell concentration, viability and TSA- and R2A-cultured strain composition. The concentration varied at an order of 10^7 cells/m^3 with dust loading (demonstrated with PM_{10}) and they had a very close correlation ($R_T^2 = 0.91$, $p < 0.01$). At the time of highest PM_{10} of 652 μg/m^3, the bacterial concentration reached 1.4×10^8 cells/m^3, which was larger than that before and after the dust event by one order. Bacterial viability, the ratio of number concentration of viable cells to total cells, was 32%–64% and smaller in the dust plume than that before the dust arrival. Bacterial strains from the culture ranged between 2.5×10^4 and 4.6×10^5 CFU/m^3 and no correlation with PM_{10} was determined. Their composition was different before and after the dust arrival according to 16S rRNA gene sequences and strains belong to *Actinomycetes* and *Firmicutes* were the majority in the dust samples.

Introduction

Bacteria as a major part of microorganisms in the air play an essential role in the development and evolution of the earth (Cao et al., 2014). Traveling in wind flows, airborne bacteria link distant ecosystems, some of them can spread pathogens and allergens that threaten public health (Griffin, 2007; Kun, 2008). Moreover, they can absorb and reflect atmospheric radiation, which in turn directly affect the energy budget in the atmosphere, and ice nucleation active bacteria also act as efficient ice nuclei to enhance cirrus formation (Bowers et al., 2009; Christner et al., 2008). Therefore, the evaluation of these environment and climate effects requires the knowledge of the concentration and composition of microorganisms in the air. In addition, the viability of airborne bacteria is the key factor for elucidating the manner by which living microorganisms spread in the air, and consequently for understanding how airborne bacteria link geographically isolated communities.

It has been proved that long-distance transport of dust in the atmosphere is an efficient way for environmental microorganisms' travel to link microbial communities in different areas (Boyd and Ellwood, 2010; Kellogg and Griffin, 2006; Rubin et al., 2011), in addition to providing nutrients from land to the marine ecosystem and helping our planet keep the balance in various aspects (Jickells et al., 2005). Evidence for the presence of bacteria with dust have been frequently obtained with gene extraction and culture-dependent methods (Chao et al., 2012; Griffin, 2008; Hua et al., 2007; Kellogg and Griffin, 2006; Schlesinger et al., 2006; Yeo and Kim, 2002). Using fluorescence microscope observation, Maki et al. (2010, 2013) and Yamaguchi et al. (2012) confirmed

* *Corresponding authors*. E-mails: hlyuan@cau.edu.cn (Hongli Yuan), dzzhang@pu-kumamoto.ac.jp (Daizhou Zhang).

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bacteria on dust particles in elevated air around Japanese islands. Hara and Zhang (2012) reported the synchronizing variation of airborne bacterial cell concentration with dust particle number concentration during the passage of a dust plume at a coastal site of the downwind area of the East China Sea, where Murata and Zhang (2014) further outlined the dynamics of airborne bacteria on the synoptic scale. Although culture-independent technique could obtain the information of the microbial concentration and viability, the culture-dependent approach can obtain the microbial strains for further study of the metabolic pathways of airborne microorganisms.

Although the concentration and composition of airborne bacteria and their evolution in space and time are essential for identifying the role and function of bacteria in the atmosphere, information on these varieties is still very poor. In particular, cell concentration of viable and non-viable bacteria associated with dust phenomena in the Asian continent, one of the largest natural dust source areas over the world, has not been reported and how dust conveys microorganisms in the air there is still unknown.

The major sources of Asia dust are the arid and semi-arid areas in the north and northwest parts of China including Taklimakan desert, Loess Plateau and Gobi desert. (Sun et al., 2001). Dust storms, occurring frequently in autumn, spring and sometime summer and winter, are considered to input large amounts of microorganisms into the air regarding the enrichment of bacteria in surface soil. Observations in dust plumes at Dunhuang, a city at the eastern edge of Taklimakan desert, revealed the abundance of bacteria in elevated dust plumes (Kakikawa et al., 2008).

The Northern Hemisphere middle latitude westerly flows distribute the dust particles eastward on local, regional and even global scales. Beijing is located in the downstream area of the dust sources and is affected by dust almost every year. The arrival of dust plumes is expected to result in the change of bacterial concentration and community in the local air. Here we report the results of airborne bacteria when a dust event passed Beijing in the end of March 2012 to show the evolution of bacterial cell concentration, viability and their correlation with dust particles within the dust-loading cyclone at the populated city.

1. Materials and methods

1.1. Sampling site and meteorological records

Aerosol samples were collected on a balcony of the Horticultural Building (about 20 m above ground; 40°01′22″N, 116°16′32″E) on the campus of China Agricultural University, Beijing, China on 30th and 31st of March in 2012. The campus is located in the northwestern outskirt area of Beijing and there are no restaurant, hospital, water and other interference factors near the sampling site. Air parcels from north or west directions are not influenced significantly by the populated area of the city. According to the dust information of the China Weather Network (http://www.weather.com.cn//), a dust plume arrived in Beijing area at about 18:00 (Beijing Standard Time, 8 hr prior to GMT) on 30th of March and vanished at about 5:00 on 31st of March.

Hourly meteorological records of temperature, pressure, relative humidity and wind near the observational site and dust forecast were obtained from the on-line report of the Beijing Meteorological Observatory. Hourly concentrations of suspended particulate matter *i.e.* $PM_{2.5}$ and PM_{10} were obtained from the on-line report of China National Environmental Monitoring Center. The PM_{10} and $PM_{2.5}$ records were used as indicators for the evolution of dust and air pollution. In addition, a sample, which was collected from 13:00 to 14:00 on 28th of March when the meteorological conditions and PM_{10} were not very different from those on the morning of 30th of March before the dust arrival, was applied to demonstrate the usual state of bacteria in the air situation under non-dust condition. During the whole observation period, the variation of $PM_{2.5}$ was relatively small in comparison with that of PM_{10} whose evolution reflected clearly the dust variation.

1.2. Sample collection

A swirling liquid impinger (BioSampler, SKC Inc., USA) was used to collect aerosol samples. To collect a sample, the BioSampler was filled with sterile 20 mL phosphate buffered saline (PBS, pH 7.4) which had been filtered through membrane filters of 0.2 μm pore size. Ambient air was pumped through the BioSampler at 12.5 L/min flow rate to achieve the best collection efficiency of bacteria (Willeke et al., 1998). The collection time for each sample was 60 min. The PBS in the sampler was checked every 20 min to make sure that the volume remained 20 mL. Any loss was compensated with sterile filtered deionized water. A negative control of the PBS was prepared together with each sample for quality assurance.

1.3. Analysis

After the collection of a sample, it was taken to laboratory immediately for analysis. The liquid sample was transferred into a 50 mL centrifugal tube which had been sterilized and washed with sterile filtered deionized water. After being fully blended on a vortex mixer, 0.5 mL was taken out and diluted with 4.5 mL sterile 0.85% NaCl solution for culture and subsequent analysis. The remained was used for the enumeration of bacterial cells with LIVE/DEAD® BacLight™ Bacterial Viability Kit (L-13152, Molecular Probes, USA).

1.3.1. Enumeration of viable and non-viable bacteria

LIVE/DEAD® BacLight™ Bacterial Viability Kit was used to enumerate bacterial cells in the samples. The kit is able to distinguish viable and non-viable bacterial cells in liquid samples (Gasol et al., 1999; Leuko et al., 2004; Gatti et al., 2006; Asadishad et al., 2011) and its effectiveness in the application to airborne bacteria has been proved in laboratory and field studies (Hara and Zhang, 2012; Murata and Zhang, 2013). The kit contained two fluorescent stains: SYTO9 and Propidium Iodide (PI). Under blue excitation ray (470–490 nm), SYTO9 labeled all bacterial cells to emit green fluorescence while PI labeled membrane-damaged bacterial cells to emit red fluorescence. Bacterial cells stained with the two stains