Atmospheric Environment 47 (2012) 20–25

Contents lists available at SciVerse ScienceDirect

Atmospheric Environment

journal homepage: www.elsevier.com/locate/atmosenv

Bacterial abundance and viability in long-range transported dust

Kazutaka Hara, Daizhou Zhang*

Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto 862-8502, Japan

ARTICLE INFO

Article history:
Received 9 July 2011
Received in revised form
18 November 2011
Accepted 19 November 2011

Keywords:
Airborne bacteria
Bioaerosol
Asian dust
Epifluorescent microscopy

ABSTRACT

Transports of bacteria in the atmosphere relate to climate and global hydrological cycles by acting as nuclei of ice-cloud formation, and affect the ecosystems and public health in the downwind ecosystems. Here we present quantitative investigations of airborne bacterial cells coupled with LIVE/DEAD BacLight assay in southwestern Japan to show that airborne bacteria were widespread with Asian dust. Total bacterial cell concentrations in dust varied between 1.0×10^6 and 1.6×10^7 cells m^{-3}, which were one to two orders higher than those in non-dusty air and were correlated with the concentrations of aerosol particles larger than 1 μm. The ratio of viable bacterial cells to total bacterial cells (viability) of bacteria in dust ranged from 16 to 40%, which was quite smaller than the viability in non-dusty air. However viable bacterial cell concentrations in dust, $2.5 \times 10^5 - 3.8 \times 10^6$ cells m^{-3}, were similar to or higher than those in non-dusty air. Dust is thus a substantial source of airborne bacterial cells as well as mineral particles. These quantitative results suggest Asian dust is one of the processes for dispersal of airborne bacteria in the global atmosphere.

1. Introduction

Atmospheric mineral dust has direct and indirect effects on the global biogeochemical cycles and the climate (Satheesh and Krishna Moorthy, 2005). Previous studies confirmed that inter-continentally transported dust (e.g. Asian and African dusts) could bring airborne bacteria to the distant regions (Echigo et al., 2005; Hua et al., 2007; Jeon et al., 2011; Kellogg and Griffin, 2006; Lee et al., 2009; Maki et al., 2010; Perfumo and Marchant, 2010). The long-range dispersal of airborne bacteria have been often concerned for their possible effects on public health and downwind ecosystems (Griffin, 2007; Hervàs et al., 2009; Ichinose et al., 2008; Kellogg and Griffin, 2006; Yukimura et al., 2009). It was also estimated that biological substances associated with dust might enhance the efficiency of dust on ice crystal formation (Pratt et al., 2009).

Bacterial communities in dust have been frequently investigated with culture methods in an attempt to study their possible effects on public health and ecosystems (e.g. Kellogg and Griffin, 2006). For example, Griffin et al. (2003) investigated airborne bacteria associated with African dust in the Caribbean atmosphere and reported that culturable bacterial concentrations in dust and non-dusty air were 105 and 13 CFU m^{-3}, respectively. Choi et al. (1997) and Jeon et al. (2011) collected air samples during Asian dust and non-dust periods in the Republic of Korea, and their results showed that culturable bacterial concentrations in dust were approximately 10^3 CFU m^{-3}, while those in non-dusty air were approximately 10^2 CFU m^{-3}. Jeon et al. (2011) also conducted DGGE analysis and 16S rDNA cloning, and demonstrated that the Asian dust affected the airborne bacterial community in terms of genetic structure and diversity. Maki et al. (2008) and Yamada et al. (2010) reported internal mixture of bacteria and mineral particles collected at 800–1200 m above the ground at the eastern edge of Taklimakan desert. These studies revealed the significance of bacterial dispersal along with dust particles.

Although previous studies have revealed that the influences of dust to the culturable bacterial communities in the air (Hua et al., 2007; Jeon et al., 2011; Kellogg and Griffin, 2006; Maki et al., 2010), only a few studies have investigated bacterial cell concentrations in dust by using culture-independent methods (Griffin et al., 2001). Furthermore, to the extent of our knowledge, there is no evaluation on the bacterial viability associated with dust. It is well-known that most bacteria in natural environments exist in a viable but non-culturable state and only 1% of the bacteria is culturable (Amann et al., 1995; Roszak and Colwell, 1987). Therefore, it is difficult to evaluate bacterial abundances in the air with culture methods (Peccia and Hernandez, 2006). Recent laboratory experiments also revealed that ultraviolet irradiation could kill viable bacteria even in an endospore state, suggesting that viable bacteria emitted into the atmosphere might become non-viable during their dispersal (Smith et al., 2011). Thus information on bacterial abundance and viability is necessary to estimate their influences on public health, downwind ecosystems and atmospheric phenomena (Griffin, 2007; Hoose et al., 2010; Ichinose et al., 2008).

* Corresponding author. Tel.: +81 (0) 96 321 6712; fax: +81 (0) 96 384 6765.
E-mail address: dzzhang@pu-kumamoto.ac.jp (D. Zhang).

1352-2310/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.atmosenv.2011.11.050

Here we report the data on bacterial abundance and viability influenced by Asian dust, which were obtained with a culture-independent approach in southwestern Japan in spring 2010. The methodology of the fluorescent microscopy coupled with fluorescent staining we used can detect culturable and non-culturable bacterial cells without cultivations, although it needs more dedicated operations and is a tedious work. The purpose of this study is to quantitatively investigate the significance of atmospheric dust in the dispersal of airborne bacteria in the global atmosphere.

2. Material and methods

2.1. Sampling site and meteorological data

Aerosol samples were collected on a balcony of a building in the campus of Prefectural University Kumamoto, Kumamoto city, Japan (32°48′N, 130°45′E; about 20 m above ground) during dust and non-dust periods between 18 February and 30 April in 2010. Kumamoto is located in southwestern Japan and Asian dust originating from the Asian continent frequently passes the site in spring.

Information on Asian dust was obtained from RIAM-CFORS (Chemical weather FORecast System) of Kyushu University, Japan (http://www-cfors.nies.go.jp/~cfors/). RIAM-CFORS is a meso-scale model developed for the simulation of regional transportations of air pollutants in East Asia (Uno et al., 2003) and the simulation results are publicly distributed by National Institute of Environment Studies of Japan (NIES, http://www-cfors.nies.go.jp/~cfors/index.html). Meteorological data including visibility and wind distributed by Japan Meteorological Agency (JMA, http://www.jma.go.jp/jma/index.html) were obtained at Kumamoto Meteorological Observatory which is approximately 5.6 km west of the sampling site. During each dust period, samples were collected every 3 h and two or three samples were intermittently collected. After dust passed away, samples were also collected for comparison.

2.2. Sample collection

A swirling liquid impinger (BioSampler, SKC Inc., USA) was applied to collect water-insoluble aerosol particles (Willeke et al., 1998). The collection efficiency of the BioSampler filled with 20 ml of water is dependent on particle size. The efficiencies for particles of 0.3 μm, 0.5 μm, 1.0 μm, and 2.0 μm are 79%, 89%, 96% and 100%, respectively (Fabian et al., 2005; Willeke et al., 1998). Re-aerosolization and cell membrane damaging rates of pre-spiked vegetative bacterial cells after 60 min collection using the BioSampler are 20% and 25%, respectively (Rule et al., 2007).

The BioSampler used in this study was preliminary siliconized with 10 nm thickness coating of dimethyl polysiloxane (L-25, Fuji systems Co., Japan) to prevent possible adherences of biological particles to the glass surface. Samples were collected with 20 ml of sterile filtered (pore size, 0.025 μm) phosphate buffered saline (PBS; 12 mM Phosphate Buffer, 140 mM NaCl, pH 7.4) in the sampler at the flow of 12.5 L min^{-1} (±0.6 L min^{-1}). To decrease the damage during the sampling process, we used sterile filtered PBS for sample collections. The collection time was 60 min for one sample. The amount of PBS was checked every 20 min and the loss was compensated by sterile filtered (pore size, 0.025 μm) deionized water in order to keep the collection efficiency. One negative control (10 ml of sterile filtered PBS in a prewashed centrifugal tube) was prepared for every sample collection. In total, 32 samples were collected in this study.

In addition, size-segregated number concentrations of aerosol particles were measured with an optical particle counter (Rion KR-12A, Rion Corporation, Japan). The counter measured particles in 6 diameter ranges of 0.3–0.5, 0.5–0.7, 0.7–1.0, 1.0–2.0, 2.0–5.0 and >5.0 μm and it counted particles in 1 L of air every 3 or 10 min. Temperatures and relative humidity were also monitored in the same place by using a thermo-hygrometer (WEATHECOM, EMPEX, JAPAN) every 5 min. In the entire sampling period, temperatures ranged from 9.1 to 21 °C and relative humidity ranged from 20 to 53%. Wind velocity during dust periods 2.1–7.1 m s^{-1} was not much different from that in non-dust periods 2.4–5.5 m s^{-1} (Data from JMA).

2.3. Analysis

To analyze bacterial cells in the collected samples, LIVE/DEAD® BacLight™ Bacterial Viability Kit (BacLight stain; L-13152, Molecular Probes, USA) was applied to stain sample liquid (Boulos et al., 1999). BacLight stain to detect bacterial cells has been already applied in many laboratory and outdoor experiments (Grey and Steck, 2001; Hernlem and Ravva, 2007; Janssen et al., 2002; Sahu et al., 2005). BacLight stain is composed of two fluorescent stains; SYTO9 and Propidium Iodide (PI). SYTO9 generally labels all bacterial cells, and PI only labels membrane-damaged bacterial cells. Bacterial cells labeled with SYTO9 emit green fluorescence. Under an epifluorescent microscope, bacterial cells stained with both stains emit yellow, orange and red fluorescence which are reflected different levels of cell membrane damage (Boulos et al., 1999), and red fluorescing cells have fully damaged cell membrane. As a result, the bacterial viability using BacLight stain is discriminated by cell membrane damage. In this study, intact bacterial cells which emit green fluorescence were identified as viable bacteria. Other bacterial cells which emit yellow, orange and red fluorescence, i.e. membrane-damaged cells were identified as non-viable bacteria. Before the outdoor experiments, tests for a sensitiveness of BacLight stain to analyze dust samples were conducted. For reference, bacterial cell counts using BacLight stain to detect bacterial cells (*Bacillus subtilis* JCM 1465^T) with or without water-insoluble mineral particles were not much different (total cells; 0.94:1.0, viable cells; 0.94:1.0, the results without the particles were revealed as 1.0). In addition, the result of total bacterial cell counts using BacLight stain to detect the cells in the mixture was nearly equal to the results using Ethidium Bromide (EB) and DAPI (BacLight:EB:DAPI = 1.0:0.93:0.96, the result using BacLight stain was revealed as 1.0).

Stock solution of BacLight stain was prepared according to the manufacture's instruction, and was stored at −20 °C. After sample collection, sample liquid was transferred into a prewashed 50 ml centrifugal tube. The inside of the BioSampler's bottom part was washed twice with 1 ml of sterile filtered deionized water. Wash solution was added to the centrifugal tube and the sample liquid was filled up to 25 ml with sterile filtered deionized water. Each sample liquid was added to 250 μl of BacLight stain, and incubated for 15 min in dark at room temperature. Sample liquid was then filtered onto a 25 mm diameter, 0.2 μm pore size black polycarbonate membrane filter (ADVANTEC MFS, Inc., Japan). After that, each filter was placed on a slide glass and covered by a cover glass. The samples were prepared in triplicate. In addition, the negative control sample was prepared with the same procedure.

For each sample collection, three subsequently-processed samples and a negative control were viewed by using an epifluorescent microscope (ECLIPSE 80i, NIKON, JAPAN) equipped with a mercury 100 w lamp. Blue excitation rays (excitation filter: 450–490 nm, emission filter: 520 nm) were applied and the microscope was operated at 1000 magnification. Bacterial cell numbers of each sample were counted from at least 20 random fields. It should be noted that the sample collection with the swirling liquid impinger and the staining process can cause cells to separate from particles such as water-insoluble mineral particles. Although bacterial cells on water-insoluble particles confirmed under the microscope were counted, we did not focus on their internal mixture. The bacterial cell counts were calibrated by subtracting the average count in the negative