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Bacterial abundance and viability in long-range transported dust

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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).

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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

control. Then, total bacterial cell concentration (viable and non-viable bacterial cells per cubic meter of air volume) and viable bacterial cell concentration (viable bacterial cells per cubic meter of air volume) were estimated with sampling air volume. The bacterial viability, which is defined by the ratio of viable bacterial cells to the total bacterial cells and applied to show the number of bacterial cells with potential living ability from a statistical perspective in this study, was also estimated. The size of a bacterium was defined as the mean of its longest and shortest scale in epifluorescent photographs with an image analyzing software (Lumina vision, Mitani Corporation, Japan).

Data obtained during different periods were separated into three groups: DUST ($n = 11$, four dust events), DUST-END ($n = 10$), and NON-DUST ($n = 11$) according to the dust situation, visibility and number concentrations of coarse particles (diameter > 1.0 μm). DUST refers to data of dust periods and visibility <20 km, DUST-END refers to data at the end of dust passage and visibility $\geqq 20$ km, and NON-DUST refers to data of non-dust periods and visibility $\geqq 20$ km.

3. Results

3.1. Enumeration

Fig. 1 shows an example of epifluorescent microphotograph of the collected aerosol samples. Under blue excitation rays, fluorescent cells labeled with BacLight stain emitted green, yellow, orange and red fluorescence. There were also water-insoluble particles which looked like mineral particles emitting yellowish fluorescence. Cells were distinguished from the particles by their fluorescent color, fluorescent intensity, morphology and size. Bacterial cells could be discriminated from other biological particles according to their morphology and size (Bae et al., 1972; Watson et al., 1977). It was found that almost all observed bacterial cells in the samples were present in a spherical shape and in the size range smaller than 1 μm. In negative controls, an average of one cell or none was identified within per observed field, indicating the ignorant artificial influence.

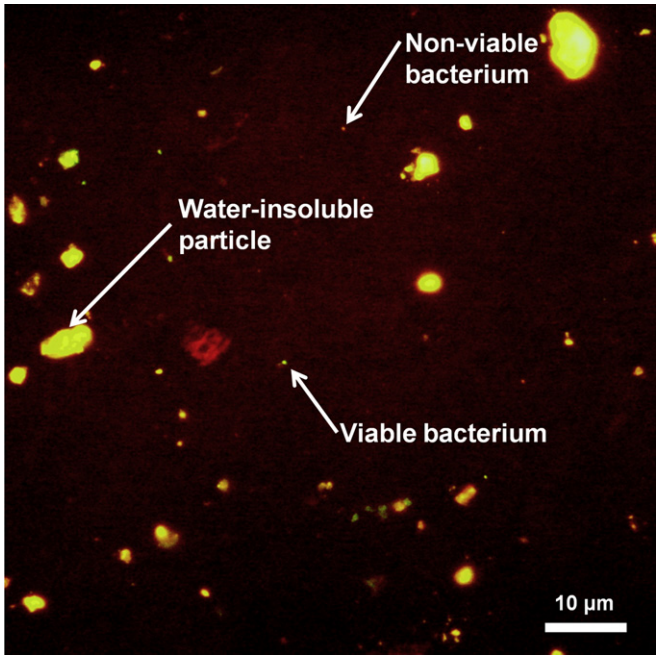

Fig. 1. Example of epifluorescence micrograph of aerosol particles. The particles were collected on 30 April in 2010 in a dust period.

3.2. Bacterial abundance vs aerosol particles

Asian dust always caused marked increases of bacterial abundances in the air. As an example, the variation of bacterial cell concentrations, particle concentrations and the simulated dust extents during the passage of a strongest dust in the whole observation period are shown in Fig. 2. The dust arrived at about 23:00 local time (Japan Standard Time: GMT + 09:00) on March 20, and the dust disappeared at about 10:00 on March 21. The variation of the number concentrations of coarse particles was adapted to demonstrate dust arrival because Asian dust always bring a large number of mineral particles larger than this size (e.g. Zhang et al., 2003). At about 03:00 on March 21 when coarse particles reached the maximum concentration 6.7×10^7 particles m^{-3}, total bacterial cell concentration was 1.6×10^7 cells m^{-3} (Fig. 2a). After that, the cell concentration decreased with decrease of coarse particles (Fig. 2ab). At about 15:00 on March 21 when the dust was ending, the cell concentration was about 6.5×10^5 cells m^{-3}, two orders lower than the maximum concentration of this dust period. The variation of bacterial abundance during dust periods closely depended on the number concentrations of coarse particles. In contrast, the figure shows that the cell concentration was not correlated with number concentrations of fine particles (0.3 μm $<$ diameter $<$ 1.0 μm), which are usually dominated by particles produced in the air via coagulations and condensations (Seinfeld and Pandis, 1998).

3.3. Bacterial cell concentrations and viability

Tables 1–3 show bacterial cell concentrations and the viability in each group. Total bacterial cell concentrations of DUST, 1.0×10^6–1.6×10^7 cells m^{-3}, were one to two orders higher than those of NON-DUST, 4.4–8.3 $\times 10^5$ cells m^{-3}, and the cell concentrations of DUST-END were intermediate. More than that, total bacterial cell concentrations of DUST correlated with the number

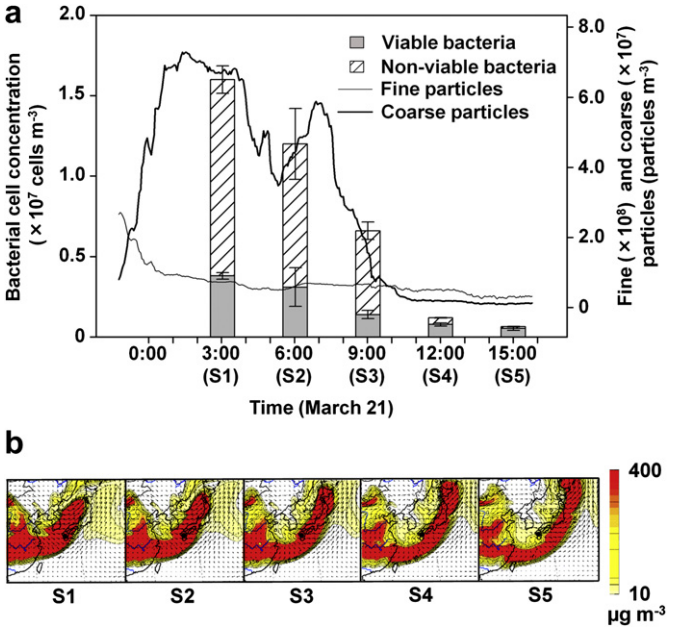

Fig. 2. Evolution of bacterial abundance and dust. (a) Time series of bacterial cell concentrations and number concentrations of fine particles (0.3 μm $<$ diameter $<$ 1.0 μm) and coarse particles (diameter $>$ 1.0 μm) on March 21. (b) Extents of the dust corresponding to the sample collections simulated by the RIAM-CFORS. The sampling site is marked by black dots.

Table 1
Bacterial cell concentrations, bacterial viability, and number concentrations of aerosol particles in DUST samples. TBCC, VBCC and BV indicate total bacterial cell concentration, viable bacterial cell concentration and bacterial viability, respectively. FP and CP indicate number concentrations of fine particles (0.3 μm < diameter < 1.0 μm) and coarse particles (diameter > 1.0 μm), respectively. The data of visibility distributed by JMA (open site: http://www.jma.go.jp/jma/index/html) was measured at Kumamoto Meteorological Observatory.

Sampling Time (JST)		Visibility (km)	TBCC ($\times 10^5$ cells m^{-3})	VBCC ($\times 10^5$ cells m^{-3})	BV (%)	FP ($\times 10^5$ particles m^{-3})	CP ($\times 10^5$ particles m^{-3})
Date	Period						
16 Mar. (Dust 1)	11:31–12:32	9	15 (±0.58)	6.0 (±0.36)	40 (±0.58)	1200 (±66)	130 (±7.9)
	14:31–15:32	12	10 (±1.3)	3.6 (±0.62)	36 (±4.7)	930 (±74)	100 (±9.4)
21 Mar. (Dust 2)	2:32–3:33	3	160 (±10)	38 (±2.0)	24 (±1.0)	740 (±9.7)	670 (±5.8)
	5:32–6:33	3	120 (±34)	31 (±12)	26 (±5.0)	610 (±64)	450 (±35)
	8:31–9:32	5	66 (±8.0)	14 (±2.6)	21 (±2.1)	640 (±62)	160 (±66)
27 Apr. (Dust 3)	14:30–15:32	10	28 (±5.2)	4.4 (±0.66)	16 (±2.1)	1200 (±60)	100 (±6.3)
	17:31–18:33	8	17 (±2.5)	2.8 (±0.30)	16 (±2.3)	1100 (±280)	85 (±18)
	20:32–21:34	15	14 (±0.95)	2.5 (±0.46)	18 (±2.6)	680 (±26)	39 (±1.8)
30 Apr. (Dust 4)	11:36–12:38	15	16 (±2.1)	5.5 (±0.71)	34 (±3.1)	670 (±46)	50 (±7.6)
	14:31–15:33	10	23 (±2.5)	5.2 (±0.99)	23 (±2.0)	670 (±28)	84 (±7.3)
	17:30–18:31	15	30 (±3.3)	6.3 (±0.86)	21 (±1.5)	680 (±31)	98 (±4.7)

concentrations of coarse particles quite well (Fig. 3). Linear regressing revealed a relation of

$$\mathrm{TBCC} = 0.9471\mathrm{CPC} - 0.2424 \quad (1)$$

with $R^2 = 0.74$ and $P < 0.001$, where TBCC is the total bacterial cell concentration (in $\log_{10}$ cells m^{-3}) and CPC is the number concentration of coarse particles (in $\log_{10}$ particles m^{-3}).

The bacterial viabilities according to the cell membrane damage in the air were quite different between dust and non-dust periods. The viability of DUST ranged from 16 to 40%, whereas the viability of NON-DUST ranged from 76 to 91%. Although non-viable bacteria were predominant in DUST samples, viable bacterial cell concentrations of DUST, 2.5×10^5–3.8×10^6 cells m^{-3}, were comparable to or higher than those of NON-DUST, 3.6–6.3×10^5 cells m^{-3}. Moreover, viable bacterial cell concentrations of DUST correlated with the number concentrations of coarse particles (Fig. 3). Linear regressing also revealed a relation of

$$\mathrm{VBCC} = 0.9868\mathrm{CPC} - 1.146 \quad (2)$$

with $R^2 = 0.82$, $P < 0.001$, where VBCC is the viable bacterial cell concentration (in $\log_{10}$ cells m^{-3}).

4. Discussion

Although culturable and non-culturable bacterial communities in dust were frequently analyzed (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), those investigations did not determine of airborne bacterial abundances and viabilities between dust and non-dust periods. Our quantitative observation indicated that bacterial abundances (mostly, non-viable bacterial cells) varied in the response to the transport of dust (Fig. 2 and Table 1). Moreover, the increases of bacterial abundances during the dust periods were closely related to the number concentrations of coarse particles (Fig. 3 and Table 1). There is a possibility that the detected bacterial cells were influenced somewhat by contaminations from local sources. But the bacterial cell concentrations were not expected to considerably exceed the concentrations in non-dusty air because the wind velocities were similar between dust and non-dust periods. Given the mean total bacterial cell concentration in non-dusty air (5.9×10^5 cells m^{-3}) as the contamination from local sources, total bacterial cell concentrations in dust (1.0×10^6–1.6×10^7 cells m^{-3}) were approximately 1.7–27 folds higher than the mean in non-dusty air, indicating long-range transported dust did carry substantial airborne bacterial cells to the areas of arrival. Actually, dust particles in the samples might inhibit the bacterial cell counts and cause underestimations of the cell concentrations in the dust samples. Re-aerosolizations of collected bacterial cells during the sample collections also caused the underestimations (Rule et al., 2007). Thus, the detected bacterial cell concentrations in dust could be considered as the lower bounds in detectable values with our experimental method. It consequently could be concluded that Asian dust was the primary source of bacterial cells at the sampling site during the dust periods.

Viable bacteria transported within dust could be protected from UV radiations and might extend their life (Griffin et al., 2003). However, the protective function of dust has not been sufficiently demonstrated with culture-independent methods. The present results indicate that the bacterial viability in long-range transported dust was considerably lower than that in non-dusty air (Tables 1 and 3). It is likely that most bacteria could not survive

Table 2
Bacterial cell concentrations, bacterial viability, and number concentrations of aerosol particles in DUST-END samples.

Sampling Time (JST)		Visibility (km)	TBCC ($\times 10^5$ cells m^{-3})	VBCC ($\times 10^5$ cells m^{-3})	BV (%)	FP ($\times 10^5$ particles m^{-3})	CP ($\times 10^5$ particles m^{-3})
Date	Period						
16 Mar.	17:30–18:31	25	9.0 (±1.3)	3.5 (±0.56)	39 (±3.8)	600 (±15)	48 (±2.0)
17 Mar.	14:31–15:32	25	9.8 (±2.4)	4.1 (±1.2)	42 (±2.6)	520 (±15)	32 (±1.0)
21 Mar.	11:30–12:31	20	12 (±1.1)	7.8 (±0.93)	65 (±3.1)	510 (±12)	20 (±1.2)
	14:30–15:31	40	6.5 (±1.4)	5.4 (±1.2)	83 (±1.2)	300 (±20)	11 (±1.0)
22 Mar.	14:32–15:33	35	15 (±2.3)	10 (±1.6)	67 (±6.4)	530 (±22)	17 (±1.4)
3 Apr.	14:33–15:35	25	11 (±1.2)	2.3 (±0.57)	21 (±5.1)	680 (±13)	41 (±1.1)
	17:33–18:35	25	9.8 (±1.1)	1.9 (±0.52)	19 (±6.1)	920 (±18)	45 (±1.2)
	20:33–21:35	20	9.6 (±1.8)	3.3 (±0.74)	34 (±4.5)	820 (±30)	30 (±1.1)
28 Apr.	14:31–15:33	30	11 (±1.4)	2.9 (±0.54)	26 (±2.1)	510 (±11)	24 (±0.80)
29 Apr.	14:31–15:32	30	9.4 (±1.7)	5.0 (±1.2)	53 (±8.7)	490 (±11)	18 (±0.78)

Table 3
Bacterial cell concentrations, bacterial viability, and number concentrations of aerosol particles in NON-DUST samples.

Sampling Time (JST)		Visibility (km)	TBCC ($\times 10^5$ cells m^{-3})	VBCC ($\times 10^5$ cells m^{-3})	BV (%)	FP ($\times 10^5$ particles m^{-3})	CP ($\times 10^5$ particles m^{-3})
Date	Period						
20 Feb.	14:40–15:41	25	6.5 (±0.28)	5.1 (±0.096)	78 (±2.6)	1200 (±130)	24 (±15)
21 Feb.	14:30–15:31	40	5.8 (±0.30)	4.9 (±0.14)	84 (±3.1)	1100 (±42)	17 (±7.3)
28 Feb.	14:31–15:32	40	6.0 (±0.46)	5.1 (±0.25)	85 (±3.5)	1000 (±110)	17 (±1.8)
11 Mar.	14:32–15:33	30	5.2 (±0.39)	4.4 (±0.25)	85 (±2.6)	950 (±53)	8.7 (±2.6)
26 Mar.	14:33–15:34	40	4.9 (±0.46)	4.1 (±0.36)	84 (±2.3)	380 (±19)	12 (±0.65)
28 Mar.	14:36–15:37	40	8.3 (±1.1)	6.3 (±0.93)	76 (±2.0)	550 (±11)	11 (±0.58)
29 Mar.	14:31–15:32	35	5.7 (±0.75)	4.6 (±0.61)	81 (±4.4)	650 (±28)	7.7 (±0.64)
8 Apr.	14:33–15:35	40	6.5 (±0.39)	5.2 (±0.19)	80 (±2.5)	500 (±16)	9.1 (±0.57)
17 Apr.	14:32–15:33	20	6.7 (±1.4)	5.2 (±1.2)	78 (±5.1)	1100 (±32)	13 (±0.64)
24 Apr.	14:46–15:47	25	4.4 (±0.34)	3.6 (±0.25)	82 (±1.0)	480 (±20)	2.4 (±0.26)
25 Apr.	14:32–15:33	50	5.3 (±1.2)	4.8 (±1.1)	91 (±2.0)	500 (±34)	3.1 (±0.35)

during their long-range transports. Atmospheric stressors such as UV radiations, low temperature and desiccation are harmful to airborne bacteria (Lighthart, 1997; Smith et al., 2011; Tong and Lighthart, 1997). The bacterial cells transported by the dust might to be exposed to the atmospheric stressors and damaged beyond their stressor-protection abilities. The viable bacterial cell concentrations in the atmospheric area would decrease during the intercontinentally dispersal. It should be noted that the results of the viability reported here were underestimated due to effects of sample collections (Rule et al., 2007) and inhabitations of dust particles in the analysis. Thus the results of the viability were the lower bounds in detectable values with our experimental method.

On the other hand, it is also noteworthy that there was a quantitative relation between coarse particles and viable bacterial cells (Figs. 2 and 3 and Table 1), suggesting that long-range transported dust also carried some viable bacteria to the areas of arrival. In fact, endospore-forming bacterial strains were frequently isolated as viable from inter-continentally transported dust (Kellogg and Griffin, 2006; Hua et al., 2007; Maki et al., 2010). Those high-tolerant bacterial strains might survive during long-range dispersal, and were efficiently dispersed by the atmospheric dust. In addition, there is no information on the viability in source regions. Long-term investigations at various sites are necessary to elucidate survival mechanisms and death processes of airborne bacteria in the atmospheric dispersal.

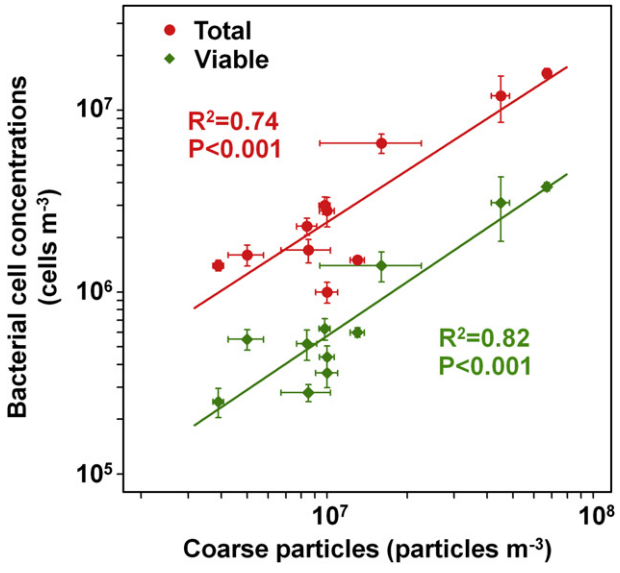

Fig. 3. Total and viable bacterial cell concentrations *vs* number concentrations of coarse particles (diameter > 1.0 μm) with standard deviations for DUST samples. Red and green lines are the linear regressions, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5. Conclusion

In this study, we conducted quantitative investigations of airborne bacteria in long-range transported dust at southwestern Japan in spring 2010. Aerosol particles were collected in dust and non-dust periods using a swirling liquid impinger and bacterial cells were microscopically quantified coupled with LIVE/DEAD BacLight assay. Results show that total bacterial cell concentrations in dust were one to two orders higher than those in non-dusty air. Furthermore, bacterial viability in dust was less than 40%, whereas that in non-dusty air was more than 76%. Although non-viable bacterial cells were predominant in dust, viable bacterial cell concentrations were comparable to or higher than those in non-dusty air. These results suggest that the long-range transport of dust in the atmosphere is likely one efficient process for the dispersal of viable and non-viable bacteria in local, regional and even global scales.

Acknowledgments

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