Assessment of composition and origin of airborne bacteria in the free troposphere over Japan

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Running Title:

Bacteria of the free troposphere over Japan

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Abstract

Long-range transport of airborne microorganisms through the free troposphere significantly impacts biological ecosystems, human life, and atmospheric processes in downwind areas. However, microbial communities in the free troposphere have rarely been investigated because the direct collection of microbial cells at high altitudes requires sophisticated sampling techniques. In this study, tropospheric air sampling was performed using a balloon and an aircraft at 800 m and 3000 m, respectively, over the Noto Peninsula in Japan (37.5°N, 137.4°E) where free tropospheric winds carry aerosols from continental areas. The air samples were collected during four different sampling periods when air masses came from desert regions of Asian continent (west samples) and from Siberia of Russia North Asia (north samples). The west samples contained higher levels of aerosols, and bacteria from the west samples grew in culture media containing up to 15% NaCl. In contrast, bacteria from the north samples could not be cultured in the same media. All isolates obtained from the NaCl-amended cultures were similar to *Bacillus subtilis* and classified as *Firmicutes*. A 16S rDNA clone library prepared from the west samples was mainly composed of one phylotype of *Firmicutes* that corresponded to the cultured *B. subtilis* sequence. A clone library prepared from the north samples consisted primarily of two phyla, i.e., *Actinobacteria* and *Proteobacteria*, which are known to dominantly inhabit low-temperature environments of North Asia. Our results suggest that airborne bacterial communities at high altitudes include several species that vary by the direction and interaction of free tropospheric winds.

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48 **Key words:** Phylogeny, Asian dust, airborne bacteria, bioaerosol, halotolerant bacteria,

49 free troposphere

1. Introduction

Bioaerosols, which include bacteria, fungi, and viruses, are transported from marine and terrestrial environments to the free troposphere and are significantly abundant in the organic carbon fraction of atmospheric aerosols (Prospero et al., 2005). Airborne microorganisms increase allergen burden causing increased incidence of asthma (Ichinose et al., 2005) and contribute to dispersion of diseases such as Kawasaki disease in humans (Rodó et al., 2011) and rust diseases in plants (Brown and Hovmøller, 2002). Moreover, bioaerosols are thought to influence atmospheric processes by participating in atmospheric chemical reactions and cloud particle formation (Pratt et al., 2012).

The bacterial species composition of the atmosphere should be investigated for understanding the characteristics of bacterial communities that are transported to long distances and influence downwind ecosystems and climates. Aerosol sampling at altitudes of 200–800 m above the ground level has demonstrated that bioaerosols are composed of several species of bacteria (Li et al., 2010). The atmosphere is a heterogeneous environment, and meteorological shifts can alter the bacterial species composition of bioaerosols. The airborne bacterial abundance and species composition at ground level in Asia (Hara and Zhang, 2012) and at 2700 m above sea level on North American mountains (Smith et al., 2012) change significantly depending on Asian dust events. However, few reports have directly investigated bacterial dynamics at high altitudes, such as the free troposphere, where long-range transported bioaerosols are abundant (Griffin 2004).

Halotolerant bacteria are tolerant to high salinity and resistant to stressors, such as high pH, extreme temperatures, and desiccation (Lippert and Galinski, 1992). Indeed, using NaCl-amendment culture techniques, viable halotolerant bacteria have been detected from bioaerosols collected at high altitudes (Maki et al., 2008). Halotolerant bacterial communities are typically common to bioaerosols transported hundreds or thousands of kilometers (Echigo et al., 2005). Some halotolerant bacteria isolated from sand dunes in the Gobi Desert were identical to bacterial species isolated in Higashi-Hiroshima, Japan, suggesting their long-range transport (Hua et al., 2007). An experimental design facilitating the isolation and identification of halotolerant bacteria at high altitudes is expected to be useful for analyzing transported bacteria through the free troposphere.

To investigate bacterial composition dynamics and the different air mass sources in the free troposphere, we collected air samples at altitudes of 800 m and 3000 m above the ground level over the edge of the Noto Peninsula, Japan. In this region, the air masses moving from continental areas to Japan can be monitored while avoiding aerosol contamination from local areas. We observed the amount of aerosols in air samples microscopically, and estimated the trajectories of air masses during the sampling periods. The viabilities of halotolerant microbial communities in air samples were evaluated using culture media amended with various NaCl concentrations. The bacterial species composition of the air samples was analyzed using clone-library analysis targeting bacterial 16S rRNA genes.

2. Materials and Methods

2.1. Sampling

Aerosol samplings were performed over Suzu City (37.5°N, 137.4°E) during four sampling periods. Suzu City is located on the northern coast of the Noto Peninsula, Japan and is the arrival site for aerosols from continental areas. A balloon was used for sampling over Suzu City from 11:00 to 12:00 on May 8, 2008 and from 10:50 to 11:50 on April 29, 2009. An aircraft was used for sampling from 14:50 to 16:50 on March 27, 2010 and from 11:50 to 13:50 on May 15, 2010. On March 27, 2010, the aircraft traveled westward from Suzu City to a distance of 150 km single way and back (Fig. 1). On May 15, 2010, from Suzu City, the aircraft traveled a distance of 150 km toward northwest, returned to Suzu City, and traveled a distance of 150 km toward northeast. The conditions of the four sampling periods are summarized in Table 1. The four samples collected on May 8, 2008; April 29, 2009; March 27, 2010; and May 15, 2010 were named A, B, C, and D, respectively.

During the sampling periods on May 8, 2008 and April 29, 2009, the air samples were collected at 800 m above the ground level using a tethered balloon (Maki et al., 2008). An air pump with a sterilized filter holder was carried by the balloon and was switched on at a specific altitude by a signal transmitted from the ground. An air sample (700 l) was collected on a sterilized polycarbonate filter (0.22- μ m pore size; Whatman, Tokyo, Japan) for 1 h. After sampling for an hour, the battery for the air pump failed at 800 m in the atmosphere.

On April 29, 2009 and May 15, 2010, aerosol samplings were performed at 3000 m above the ground level using an aircraft with a 25-mm-diameter hole at the top. A

sterilized sampling tube (1.5 m in length) was inserted into the hole with one end of the tube projecting outside. The other end of the tube was connected to a sterilized filter holder (In-Line Filter Holder, 47 mm; Millipore, Tokyo, Japan) in the sampling device. Given the length and curvature of the sampling tubes used, a loss of particles exceeding 0.2 μm in diameter should be considered (Hermann et al., 2001) and less than 5% of particles were lost in this sampling, but the loss could be neglected. Air samples (1400 l) were collected on each sterilized polycarbonate filter for 2 h. The samples were collected on two filters during each sampling period.

2.2. Characteristics and trajectories of air masses

Air quality and atmospheric data in the free troposphere were obtained from the Wajima Meteorological Observatory of the Japan Meteorological Agency, which is located at a distance of 100 km from the sampling sites. Environmental data were collected using a radiosonde at 3:00 a.m. At altitudes of approximately 3000 m, information regarding weather conditions, temperatures, relative humidities, wind speeds, and wind directions were obtained for comparative analyses of air masses (Table 1). The potential temperature (PT) on March 27, 2010 suggested the presence of typical free tropospheric air. It also suggested that slight cold air had activated small-scale convection, causing the sky to become cloudy. PT on May 15, 2010 indicated that weak anticyclones may be prevalent in this region. Changes in aerosol transportation are primarily controlled by the prevailing air flowing from China, the anticyclonic circulation over the north-central East China Sea, and the subsiding continental outflow air with low-level transport over Korea and Japan. Occasionally, a

cyclonic flow originates from the western North Pacific or from the East China Sea contributing to the atmospheric conditions in this region. Therefore, isentropic back trajectory analysis was applied to understand the primary transport patterns affecting aerosols collected on March 27, 2010 and May 15, 2010.

To track the transport pathways of air masses, 72-h backward trajectories were calculated using the NOAA Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model (<http://www.arl.noaa.gov/HYSPLIT.php>). The location of the backward trajectory start point was used as the sampling location for this study (37.5°N, 137.4°E) with altitudes of 2900; 3000; and 3100 m above the ground level for estimating the accurate trajectories of air masses in the free troposphere.

2.3. Microscopic analysis of particle abundance

Within 2 h of sampling, aerosols were washed off the filters by shaking with 10 ml of sterilized water containing 0.9% (w/v) NaCl. After washing, aliquots of 8 ml were fixed with paraformaldehyde at a final concentration of 1%. Samples were stained with 4, 6-diamidino-2-phenylindole (DAPI) at a final concentration of 0.5 µg/ml for 15 min and filtered through a polycarbonate filter (0.22-µm pore-size; Whatman) stained with Sudan Black (Russell et al., 1974). After the filter was placed on a slide on a drop of low-fluorescence immersion oil, a second drop of oil was added and the coverslip was placed. The prepared slides were observed under an epifluorescence microscope (Olympus, Tokyo, Japan) equipped with a UV excitation system. A filter transect was scanned, and mineral particles, yellow particles and bacterial cells on the transect were counted. The detection limit of aerosols was below 5×10^5 particles/m³ of air.

2.4. Physiological experiments

The remaining 2 ml of aerosols obtained after washing the filters with 10 ml of 0.9% (w/v) NaCl solution were used as cultivation spike in media containing various NaCl concentrations to assess the viabilities of halotolerant bacteria in the air samples. The washed solution (0.5 ml/sample) was inoculated into 19.5 ml of trypticase soypeptone (TS) liquid medium (17 g trypticase peptone, 5 g phytone peptone, 2.5 g K₂PO₄, and 2.5 g glucose in 1 l pure water) containing NaCl at final concentrations of 0%, 3%, 10%, or 15% (w/v). TS medium has often been used for detecting microorganisms from air samples. Microorganisms in the air samples were cultivated in the media at 20°C in the dark. Microbial growth was estimated every 2 days by measuring the absorbance at 550-nm.

2.5. Identification of bacterial isolates by amplifying 16S rRNA sequences

After 12 days of incubation, 1 ml of the microbial culture was used for bacterial isolation by the spread-plate method using TS agar plates. After the bacterial isolates were incubated in 10 ml of TS medium for 3 days, the bacterial cells were collected by centrifugation at 20000 × *g* for 5 min. Genomic DNA (gDNA) was extracted from the bacterial cell pellets using SDS, proteinase K, and lysozyme, as described previously (Maki et al., 2008). gDNA was purified by phenol–chloroform extraction, chloroform extraction, and ethanol precipitation. Fragments of 16S rDNA (approximately 1450 bp) were amplified from the extracted gDNA by PCR using the following oligonucleotide primers: 27F, 5'-AGA GTT TGA TCM TGG CTC AG-3'; 1492R, 5'-GGY TAC CTT

GTT ACG ACT T-3' (Maidak et al., 1997). Thermal cycling was performed using a Program Temp Control System PC-700 under the following conditions: denaturation at 94°C for 1 min, annealing at 56°C for 2 min, and extension at 72°C for 2 min for 30 cycles. PCR amplicons were purified by phenol–chloroform extraction, chloroform extraction, and ethanol precipitation. The amplicons were sequenced using a Dye DeoxyTM Terminator Cycle Sequencing Kit (ABI, CA, USA) and an ABI Prism 373A DNA Sequencer according to the manufacturer's recommended protocols. The primers 27F and 1492R were used as the sequencing primers. The amplicon sequences were searched against the DNA Data Bank of Japan (DDBJ) using BLAST. A phylogenetic tree including all sequences was constructed according to the neighbor-joining algorithm using TreeViewPPC (Saitou and Nei, 1987).

2.6. Bacterial 16S rDNA clone libraries

The 10 ml filter wash solution was used to estimate bacterial species composition by clone library analysis targeting 16S rDNA. gDNAs from the solution were extracted and purified as described in Section 2.5. Fragments of 16S rDNA (approximately 1450 bp) were amplified from the extracted gDNA by PCR using the following oligonucleotide primers: 27F and 1492R. Thermal cycling was performed using the Program Temp Control System PC-700 under the following conditions: denaturation at 95°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min for 30 cycles. PCR amplicons corresponding to 16S rDNA fragments were cloned into *Escherichia coli* using a commercially available vector plasmid with a TA Cloning Kit (Invitrogen, CA, USA) according to the manufacturer's protocol. Some clones were

obtained for each sample, and the sequences were determined as described in Section 2.5, except that M13 forward primer was used as the sequencing primer.

2.7. Accession numbers

DDBJ accession numbers for the 16S rDNA sequences determined in this study are shown in Table 2.

3. Results

3.1. Microscopic observation of aerosols

DAPI-stained mineral particles collected at 3000 m on March 27, 2010 were observed by epifluorescence microscopy as relatively large particles emitting white–blue self-fluorescence with a diameter of $>5\ \mu\text{m}$ (Fig. 2). DAPI-stained bacteria were observed as coccoid-like particles with a diameter of $<1\ \mu\text{m}$ and bright-blue fluorescence. They were attached to the mineral particles. Yellow fluorescent particles, potentially organic matter, were observed to range in diameter from $0.2\ \mu\text{m}$ to $10\ \mu\text{m}$.

DAPI-stained samples A and C included substantial concentrations of mineral and yellow fluorescent particles (approximately 10^6 particles/ m^3 ; Table 1), as observed by epifluorescence microscopy. The total densities of bacterial cells in samples A and C were $(18.4 \pm 3.2) \times 10^6$ particles/ m^3 and $(2.28 \pm 0.83) \times 10^6$ particles/ m^3 , respectively. In contrast, samples B and D contained particle concentrations below the detection limit (i.e., 5×10^5 particles/ m^3) for microscopic observation.

3.2. Physiological cultures

Microbes in samples A and C grew in TS liquid media containing 0%, 3%, and 10% NaCl, as indicated by a rapid increase in the absorbance at 550 nm to >95 (approximately 4×10^7 cells/ml) within 5 days of incubation and fluctuated in the range 10–420 during the experimental period (Fig. 3a, c). Samples A and C, amended with 15% NaCl, demonstrated minimal microbial growth from the 8th and 4th day, respectively, and the absorbance gradually increased to >10 during the experimental period. These results indicated that microorganisms capable of tolerating up to 15% NaCl maintained their viabilities in samples A and C. In contrast, no microbial growth was observed from samples B and D in any culture medium during the incubation period (Fig. 3b, d). Uninoculated culture medium in all experiments indicated no microbial growth during the experimental period, suggesting that there was no microbial contamination.

Colonies on the agar plates with the NaCl-amended cultures of samples A and C were isolated on the basis of colony formation and colors. Consequently, a total of 8 isolates were obtained from each NaCl-amended culture. Sequencing of 16S rDNA indicated that all the 8 isolates belonged to *Firmicutes* and were closely related to *Bacillus subtilis* with >99.9% similarity (Table 2).

3.3. Comparison of 16S rDNA clones

The 16S rDNA fragments in the air samples were amplified by PCR using primers targeting eubacterial 16S rDNA. The PCR amplicons were cloned into *E. coli*, and a total of 201 clones including eubacterial 16S rDNA fragments were obtained from the

four samples. Sequences of the 16S rDNA clones indicated that the bacterial populations were divided into 10 phylotypes (sequences with >98% similarity; Table 2). The majority of phylotypes recovered from the four samples belonged to the phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* that are typically well represented in 16S rDNA clone libraries generated from terrestrial and marine environments.

More than 85% of clones derived from samples A and C belonged to *Firmicutes*, and all *Firmicutes* sequences corresponded to a single phylotype that was closely related to *B. subtilis* (>99.7% similarity). Isolates obtained from NaCl-amended cultures were identical to *B. subtilis* (Fig. 4). The complete 16S rDNA sequences of isolated *B. subtilis* indicated high similarities (>99.7%) with *B. subtilis* detected hundreds of meters above the Taklamakan Desert, China and from dust layers in the snow cover of Mount Tateyama, Japan (2450 m). Sample C also included other bacterial species assigned to the phyla *Bacteroidetes* and *Proteobacteria* (Fig. 4). Of these, a phylotype comprising four clones belonging to *Bacteroidetes* was related to *Owenweeksia hongkongensis* at low similarity (<88.5%). The one remaining clone belonged to the family *Xanthomonadaceae* in *Gammaproteobacteria* and was closely related to *Pseudoxanthomonas byssovorax* (99.3% similarity).

All 20 clones derived from sample B were affiliated with *Proteobacteria*, including *Alphaproteobacteria* and *Betaproteobacteria*. The *Alphaproteobacteria* included 10 clones (50%) that were identical to *Sphingomonas rhizogenes* (100% similarity; Table 2, Fig. 4). In *Betaproteobacteria*, 7 clones (35%) were closely related to *P. fluorescens* (100% similarity) and 3 clones (15%) were similar to bacterium P618 (100%

similarity).

Isolates from sample D were dominated by *Proteobacteria* and *Actinobacteria* sequences. Of the 71 clones derived from sample D, 55 belonged to *Proteobacteria*. Forty-two (55%) of the isolates were related to *Variovorax paradoxus* (99.7%–100% similarity), and 13 (18%) were closely related to *Methylobacterium* spp. (99.7%–100% similarity; Table 2, Fig. 4). *Actinobacteria* included 13 clones (18%) that belonged to *Brevibacterium* and were related to *Brevibacterium* sp. SA312 (>99.8% similarity).

3.4. Transport trajectory

Backward trajectory analysis indicated that the air-mass sources could be classified into two types across the four sampling periods. The air masses sampled on May 8, 2008 and March 27, 2010 from the desert area of Asia had passed over the industrial area in China and across the Sea of Japan (Fig. 5a, c). In contrast, the air masses sampled on April 29, 2009 and May 15, 2010 were from North Asia areas, such as eastern Siberia and the Japanese island, Hokkaido and had passed along the Sea of Okhotsk to Suzu City (Fig. 5b, d).

4. Discussion

4.1. Bioaerosols in the free troposphere

Bioaerosols originating from Asia are dispersed in downwind regions such as Korea and Japan by the prevailing westerly winds in the middle latitudes and are sometimes carried to the Pacific Ocean (Iwasaka et al., 2009). Long-range transport of

microorganisms contributes to microbial dispersal and significantly impacts ecosystems, human health, agricultural productivity, and climate in downwind areas (Jaenicke, 2005 ; Brown and Hovmøller, 2002). In this study, epifluorescence microscopy demonstrated that aerosols collected at 3000 m contained large particles attached with microorganisms, such as bacteria (Fig. 2). The bacterial populations were possibly transported from other regions and dispersed to Japanese environments. Because atmospheric dispersion transports microorganisms to long distances, airborne bacterial composition should be compared among air masses from different continents to better understand bacterial dynamics in downwind regions (e.g., Noto Peninsula, Japan).

Samples A and C came from desert regions of Asia, whereas samples B and D came from Siberia and Hokkaido (Fig. 5). Samples A and C contained greater aerosol concentrations than samples B and D and included significant amounts of minerals, potential organic components, and bacterial particles (Fig. 2, Table 1). DAPI-stained particles with yellow fluorescence have been reported to resemble organic materials originating from proteins and other microbial cell components (Mostajir et al., 1995). The large sizes of minerals and organic particles could shelter bacterial cells against environmental stressors such as UV irradiation and desiccation.

Dust events have been reported to increase the number of airborne bacteria in correspondence with the number of mineral particles (Hara and Zhang, 2012; Prospero et al., 2005). In East Asia during spring and summer, the prevailing westerly winds constantly carry dust particles, creating weak Asian dust events at 4000 m (Iwasaka et al., 1988). During the May 8, 2008 and March 27, 2010 sampling periods, the westerly wind was believed to carry bioaerosol-associated mineral particles to high altitudes

above Suzu City. In fact, the Ozone Monitoring Instrument (<http://jwocky.gsfc.nasa.gov/>) and light detection and ranging (lidar) measurements at Toyama City, Japan, revealed that dust particles were transported to Japan on May 8, 2008 (Maki et al., 2010).

As described in Section 2.1, sampling losses of particles exceeding 0.2 μm in diameter could be neglected in this sampling. Therefore, our discussions of bacterial species composition are accurate for particles with diameters of 0.2-2.0 μm . Few investigators have examined the ratio between particle size and bacterial species composition. An understanding of this relationship can lend insight to bacterial transportation processes on the global scale, and further investigation is required on this relationship.

4.2. Bacterial communities from western and northern areas

Samples A and C included viable halotolerant bacteria that grew in culture media containing up to 15% NaCl (Fig. 3). All isolates obtained from the NaCl-amended cultures were identical to *B. subtilis* and were abundant in the 16S rDNA clone libraries from samples A and C (Table 2). Denaturing gradient gel electrophoresis analysis using PCR products demonstrated that all bacteria grown in the NaCl-amended cultures corresponded to *B. subtilis* (data not shown). *Bacillus* spp. form resistant endospores to enhance their survival in the atmosphere (Nicholson et al., 2000). Halotolerant bacteria identified as *B. subtilis* were dominantly associated with mineral particles collected at high altitudes above the Taklamakan Desert (Maki et al., 2008) and from accumulated aerosols in the snow cover of Mount Tateyama (Maki et al., 2011). Species related to *B.*

subtilis were isolated from sand samples of the Gobi Desert (Hua et al., 2007) and dominates the surface air of Saul City during Asian dust events (Jeon et al., 2011). From a free-tropospheric sampling on the North American mountains, isolates of *Bacillus* spp. were mainly obtained from air samples carried by Asian dust events (Smith et al., 2012). Presumably, halotolerant *B. subtilis* in samples A and C maintained their viability at high altitudes and were carried from continental desert areas by westerly winds.

The clone libraries obtained from samples B and D were mainly dominated by *Proteobacteria* and/or *Actinobacteria* (Table 2, Fig. 4). Terrestrial bacteria in Siberia primarily belonged to *Proteobacteria* (Zhou et al., 1997). In addition, marine bacterial communities in the Antarctic Sea are primarily composed of *Proteobacteria* (Brinkmeyer et al., 2003). The *Proteobacterium*, *V. paradoxus*, that was predominant in sample D is abundant in the snow cover of Mount Tateyama (Segawa et al., 2005) and has been isolated from a Greenland glacier ice core (Sheridan et al., 2003). *Actinobacteria* sequences from sample D were dominated by *Brevibacterium* spp. that originated from soil samples in the Arctic and Antarctica, as confirmed against DDBJ. *Pseudomonas* spp. identified in sample B were 100% similar to *P. fluorescens* originating from polar regions (Berg et al., 2009). *Sphingomonas* sp. from sample B was identical to *S. rhizogenes* and *Sphingomonas* spp. detected from Lake Baikal, Siberia, and Antarctica (Dieser et al., 2010). Members of *Sphingomonas* were often included in marine bacterial communities in North Asia and polar regions (Gloeckner et al., 2000). Several of the bacterial communities in samples B and D could have been carried to high altitudes by the north wind that originated above low-temperature environments in North Asia.

The 16S rDNA clone libraries from samples A and C were composed of bacterial species belonging to *Firmicutes* and/or *Bacteroidetes*. In contrast, the clone libraries from samples C and D primarily included sequences of *Proteobacteria* and/or *Actinobacteria* (Fig. 4). Thus, the bacterial species compositions of the four sampling dates were stratified into two different types depending on the air mass sources (Gobi Desert and North Asia). We previously found that when atmospheric wind directions in Kanazawa City, Japan changed from west to north following an Asian dust event during the first week of May 2011, airborne bacterial compositions at 10 m above ground changed from primarily *Firmicutes* (*B. subtilis*) to *Proteobacteria* (data not shown). These results suggest that bacterial communities at high altitudes varied among the four sampling periods during which the air mass sources were from the west and north. However, *Proteobacteria* detected in samples B and D did not overlap (Fig. 4). Since *Proteobacteria* are represented sparsely in the free troposphere, they may be easily affected by migrations of bacterial communities from several terrestrial and marine environments along various air-mass trajectories.

4.3. Influences of bacterial communities on ecosystems and human health

The air samples collected from the free troposphere included several bacterial species in the phyla *Firmicutes* and *Proteobacteria* that are often associated with plant growth, human life, and organic matter cycles. Although most bacterial species detected from air samples are non-pathogenic, a dominant *B. subtilis* strain in clinical contaminants has been described as an opportunistic pathogen (Thomas and Whitte, 1991). Isolates from sample B comprising a minor phylotype of *Gammaproteobacteria*

were closely related to clinical and harmful pathogens, such as *Hafnia* and *Salmonella* spp. (Ridell et al., 1994; Wang et al., 1997). Bacterial species of the genera *Methylobacterium*, *Sphingomonas*, *Variovorax*, and *Bacillus* dominated the air samples and were often found to be associated with leaf surfaces and in the rhizosphere (Idris et al., 2004; Anda et al., 2011). *V. paradoxus* and *Bacillus* spp. have been reported to promote plant growth (Maimaiti et al., 2007; Yadav et al., 2011), whereas *Bacillus* includes several species of plant pathogens (Yoshida et al., 2001). Bacterial populations on leaf surfaces or in the rhizosphere may become aerosolized from grass mowing and can disperse to other regions. The *Variovorax*, *Bacillus*, and *Pseudomonas* sequences dominating the air samples in this study were related or identical to the bacterial species mineralizing organic matters, such as cellulose, and contributed to the carbon cycle in terrestrial environments (Das and Mukherjee, 2007; Ulrich et al., 2008). Some strains of *B. subtilis* ferment organic matters and are useful for the production of Japanese health foods such as *natto* (Ashiuchi et al., 1998). The long-range dispersal of bacteria has positive and negative implications for human societies, plant growth, and microbial ecosystems.

Bacteria classified as *Xanthomonadaceae* and *Pseudomonadaceae* that are detected in air samples as minor species facilitate ice-nucleation for cloud formation in the atmosphere (Pratt et al., 2009; Morris et al., 2008). Clone library analyses of air samples over high mountains have revealed that ice nuclei-forming bacteria were minor components in the atmosphere, accounting for <1% of total clones (Bowers et al., 2009). Members of *Xanthomonadaceae* were rare in the clone library from sample C. These results suggest that air masses in the free troposphere contain a low number of bacterial

species that possess ice-nucleating activities.

5. Conclusion

To best of our knowledge, this is the first study to compare bacterial communities in the atmosphere at 800 m and 3000 m over Asia during four sampling periods when the air masses were transported from two different sources (the Gobi Desert and North Asia). The air masses originating from the Gobi Desert included halotolerant bacteria dominated by *B. subtilis* strains that are believed to have been carried by Asian dust events. In contrast, air masses originating from North Asia did not include any halotolerant bacteria and were primarily composed of *Proteobacteria* and *Actinobacteria*. It is possible that bacterial communities at high altitudes exhibit significantly different dynamics depending on the origin of the air mass. Further investigations are required to establish correlations between bacterial species composition and air mass sources. An understanding of the relationships between aerosol sizes and bacterial species and amounts is essential for predicting the dispersal conditions of bioaerosols around downwind environments.

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590

Figure Legends

Fig. 1 Aircraft flight routes during the sampling periods: 14:50-16:50 on March 27, 2010 (solid line) and 11:50-13:50 on May 15, 2010 (dotted line).

Fig. 2 Epifluorescence micrograph of mineral particles attached to bacterial particles (a) and yellow (organic) particles (b). The bioaerosols were collected on March 27, 2012. Arrows indicate bacterial cells. All photomicrographs were taken at a magnification of $\times 1000$. (scale bar = $5\mu\text{m}$).

Fig. 3 Microbial growth from samples A–D collected on May 8, 2008 (a), April 29, 2009 (b), March 27, 2010 (c), and May 15, 2010 (d), respectively, in media amended with NaCl at concentrations of 0% (squares), 3% (circles), 10% (triangles), and 15% (diamonds). All experiments were performed in five replicate tubes.

Fig. 4 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained from the clone libraries from air samples and from isolates grown in NaCl-amended media. The phylogenetic tree was calculated from a dissimilarity matrix of an approximately 330-bp alignment (*Escherichia coli* numbering 153 to 482) using a neighbor-joining algorithm. The sample information and the accession number of each reference sequence are given in parentheses. Open circles at branch points indicate that bootstrap values obtained by neighbor-joining analysis exceeded 50% (after 1000 resamplings).

614

615 Fig. 5 Three-day backward trajectories of aerosols that arrived at Suzu City on May 8,
616 2008 (a), April 29, 2009 (b), March 27, 2010 (c) and May 15, 2010 (d).

617

Table 1 Sampling dat, meteological conditions, and particle concentrations during the sampling periods.

Air sample name		A	B	C	D
Sampring information	Sampling date	May 8th, 2008	Apr 29th, 2009	Mar 27th, 2010	May 15th, 2010
	Collection time	11:00 – 12:00 (1h total)	10:50 – 11:50 (1h total)	14:50 – 16:50 (2h total)	11:50 – 13:50 (2h total)
	Sampling method	baloon	baloon	aircraft	aircraft
	Sampling location ^{*1}	800m	800m	3000m	3000m
Troposheric meteological condition	Observed weather conditions	Clear	Clear	Cloudy skies	Clear
	Temperature (°C)	0	-6.2	-18.4	1.2
	% Relative humidity	100	3	85	4
	Predominat wind direction	W	NNE	W	NNE
Concentrations of particles (10 ⁶ particles /m ³) ^{*2}					
	Mineral particles	8.84 ± 1.94	N.D ^{*3}	1.05 ± 0.79	N.D ^{*3}
	Yellow particles	6.95 ± 1.45	N.D ^{*3}	1.93 ± 0.70	N.D ^{*3}
	Bacterial cells	18.4 ± 3.2	N.D ^{*3}	2.28 ± 0.83	N.D ^{*3}

^{*1} Height above the ground.
^{*2} (particles/m³) indicates (particles per m³ of air).
^{*3} Particles were not detected under microscopic observation.

Table 2 Phylogenetic affiliation of sequences of 16S rDNA clones.								
Air sample name	Numbers of Clones or strains ^{*1}	Names of sequences ^{*2}	Conditon	Length (bp)	Category	GenBank accession no.	Closest relative	Similarity (%) ^{*3}
A	65	SzDc-08May-1	directly extracted DNA	1431	<i>Firmicutes</i>	AB749769	<i>Bacillus subtilis</i> (JQ762447)	99.7 – 100
B	10	SzDc-09Apr-1	directly extracted DNA	510	<i>Alphaproteobacteria</i>	AB609064	<i>Sphingomonas rhizogenes</i>	100
	7	SzDc-09Apr-2	directly extracted DNA	465	<i>Gammaproteobacteria</i>	AB609063	<i>Pseudomonas fluorescens</i>	100
	3	SzDc-09Apr-3	directly extracted DNA	500	<i>Gammaproteobacteria</i>	AB609067	Bacterium P618 (JX12010)	100
C	60	SzDc-10March-1	directly extracted DNA	1452	<i>Firmicutes</i>	AB740157	<i>Bacillus subtilis</i>	99.8
	4	SzDc-10March-2	directly extracted DNA	1394	<i>Bacteroidetes</i>	AB740158	<i>Owenweeksia hongkongensis</i>	88.4 – 88.5
	1	SzDc-10March-3	directly extracted DNA	1349	<i>Gammaproteobacteria</i>	AB740159	<i>Pseudoxanthomonas byssovorax</i>	93.6
D	42	SzDc-10May-1	directly extracted DNA	509	<i>Betaproteobacteria</i>	AB769480	<i>Variovorax paradoxus</i>	99.8 – 100
	13	SzDc-10May-2	directly extracted DNA	482	<i>Alphaproteobacteria</i>	AB769478	<i>Methylobacterium</i> sp. SKJH-1	99.8 – 100
	13	SzDc-10May-3	directly extracted DNA	526	<i>Actinobacteria</i>	AB769479	<i>Brevibacterium</i> sp. SA312	99.8 – 100
	3	SzDc-10May-4	directly extracted DNA	476	<i>Firmicutes</i>	AB769477	<i>Streptococcus australis</i>	100
A	4	08Szi-1	<15%NaCl	1426	<i>Firmicutes</i>	AB749540	<i>Bacillus subtilis</i> (AY553094)	100
C	3	10Szi-1	<10%NaCl	1409	<i>Firmicutes</i>	AB740155	<i>Bacillus subtilis</i> (GU826163)	100
	1	10Szi-4	15%NaCl	1426	<i>Firmicutes</i>	AB740156	<i>Bacillus subtilis</i> (HQ425655)	99.9

*1 The numbers of the clones in 16S rDNA clone libraries and the strains of culture isolates.
*2 Isolates from the NaCl amended cultures are named as the Szi serie . Clones of 16S rDNA library were named as the SzDc serie.
*3 Similarity value between each isolate and the closest relative in databases.

Fig. 1 T.Maki et al.

Fig. 2 T.Maki et al.

Fig. 3 T.Maki et al.

Fig. 4 T.Maki et al.

(a) NOAA HYSPLIT MODEL
Backward trajectories ending at 0200 UTC 08 May 08
GDAS Meteorological Data

(b) NOAA HYSPLIT MODEL
Backward trajectories ending at 0300 UTC 28 Apr 09
GDAS Meteorological Data

(c) NOAA HYSPLIT MODEL
Backward trajectories ending at 0600 UTC 27 Mar 10
GDAS Meteorological Data

(d) NOAA HYSPLIT MODEL
Backward trajectories ending at 0300 UTC 15 May 10
GDAS Meteorological Data

Fig. 5 T.Maki et al.

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