Investigation of bacterial effects of Asian dust events on the human health and ecosystem in downwind area

Graduate School of Pharmaceutical Sciences
Osaka University
Jonguk Park

General Introduction

Aeolian dust events are a natural phenomenon that primarily occur in arid and semi-arid regions, such as deserts and areas with loess. In this phenomenon, sand particles are lofted by ascending air currents, and transported over long distances (thousands of kilometers). Around 0.5–5.0 billion tons of aeolian dust particles are transported worldwide each year (1). Major aeolian dust events originate from the Sahara and Sahel deserts (African dust), the Australian deserts (Australian dust), as well as the Taklamakan and Gobi deserts, and the Loess Plateau (Asian dust). It is estimated that approximately four million tons of Asian dust fall on Japan each year (2), which is located 3,000–5,000 km away from its source regions. Asian dust sometimes even reaches North America, more than 15,000 km from its source regions (3).

Atmospheric currents are one of the main vehicles for bacterial migration, and it has been reported that bacteria adhering to aeolian dust particles may impact human health and ecosystems (4,5). Recently, bio-imaging directly observed bacteria attached to aeolian dust particles (6), prompting several investigations to evaluate the potential health effects of long-distance transport of bacteria by Asian dust. These studies carried out analyses of bacterial community composition (7), abundance and viability estimates (8), as well as investigations of atmospheric halotolerant bacterial communities (9). However, most studies have only investigated the effects of Asian dust during the dust storm season.

The aim of this study is to evaluate the effect of wind-borne bacteria on human health and ecosystems in downwind areas. Therefore, in this study, outdoor bacterial abundance and community composition were determined using 16S rRNA gene-targeted quantitative polymerase chain reaction (PCR) and amplicon sequencing. Sequences were compared from samples collected on Asian and non-Asian dust days (2013–2015; 44 samples over four seasons). This study also monitored the bacterial abundance and community composition in

outdoor aerosol samples from Beijing, China, which is closer to the Asian dust source regions, and compared sequences from these samples with the results obtained from a distant region (Osaka, Japan) to identify changes in the atmospheric bacterial community along a downwind trajectory from the dust source region.

Chapter 1

Investigation of bacterial effects of Asian dust events through comparison with seasonal variability in outdoor airborne bacterial community

1.1 Introduction

Previous studies of aeolian dust using culture-dependent methods have reported the airborne transport of pathogens and their resultant health effects (10,11). However, community analyses using culture-independent methods have confirmed that aeolian dust transported not only pathogenetic bacteria, but also phylogenetically diverse bacteria (6,9,12-14). In addition, most studies have been conducted only during Asian dust events (March to June) (6,9,12,14), even though the bacterial abundance and community composition of aerosols in outdoor environments are thought to be affected by seasonal and weather-related variations (15-19). To assess the effects of bacteria transported with aeolian dust on public health and the environment, bacterial variations should be evaluated by long-term monitoring.

Therefore, the present study was conducted to investigate the effects of bacteria transported by Asian dust events on humans and the ecosystem based on outdoor aerosol samples collected on both Asian dust and non-Asian dust days from 2013 to 2015. We analyzed variations in bacterial abundance and bacterial community composition on non-Asian dust days to understand variations in the local airborne bacterial community. We then investigated airborne bacterial community characteristics following Asian dust events through comparison with seasonal bacterial community variability on non-Asian dust days and changes in the bacterial community composition on Asian dust days. Airborne bacterial abundance and community were determined by 16S rRNA gene-targeted quantitative PCR and amplicon sequencing, respectively.

1.2 Materials and Methods

1.2.1 Sample collection

Aerosol samples were collected from the rooftop of a building (*ca*. 20 m in height) at Osaka University in Osaka, Japan (latitude: N34°9′1.89″, longitude: E135°31′15.61″) using a high-volume air sampler (HV500R [SIBATA, Saitama, Japan]). The sampling point was located approximately 12 km from a major downtown area, and there were no industrial plants, agricultural fields or superhighways around the sampling point. Air samples were collected on 0.6 μm pore-size glass fiber filters at 500 L min^{-1}. Aerosol particles from a total of 100 m^3 of ambient air were collected during each sampling event (sampling time: 200 min). Furthermore, particle size distribution (> 0.3 μm, > 0.5 μm, > 0.7 μm, > 1.0 μm, > 2.0 μm and > 5.0 μm) was determined using a particle counter (ARTI HHPC-6 [HACH, Tokyo, Japan]). Overall, 44 samples were obtained on different days between May 2013 and April 2015, including Asian dust days (20 May 2014, 27–31 May 2014, 1–3 June 2014 and 18 April 2015), all four seasons and after rainy days (Table 1.1). The occurrence of atmospheric Asian dust was confirmed using information from the Japan Meteorological Agency, LIDAR (Light Detection and Ranging) data from the Ministry of the Environment, Japan (http://www-gis5.nies.go.jp/eastasia/Dust Lider.php), and visibility at the sampling location (Fig. 1.1).

The geographic origins of Asian dust collected in this study were determined by back trajectory analysis (http://ready.arl.noaa.gov/HYSPLIT.php), and we confirmed that the origin of all Asian dust samples was the Gobi Desert.

1.2.2 DNA extraction

Aerosol samples collected on the glass filter were pulverized by bead-beating (EZ-Beads [EZ, Tokyo, Japan], 4,800 rpm, 90 sec.). DNA was then extracted and purified using the method described by Tsai and Olson (20). Extracted DNA was subsequently purified using a Wizard DNA Clean-Up System kit (Promega, Madison, WI, USA) and eluted with 50 μL of TE buffer (10 mM Tris-HCl and 1 mM EDTA [pH 8.0]).

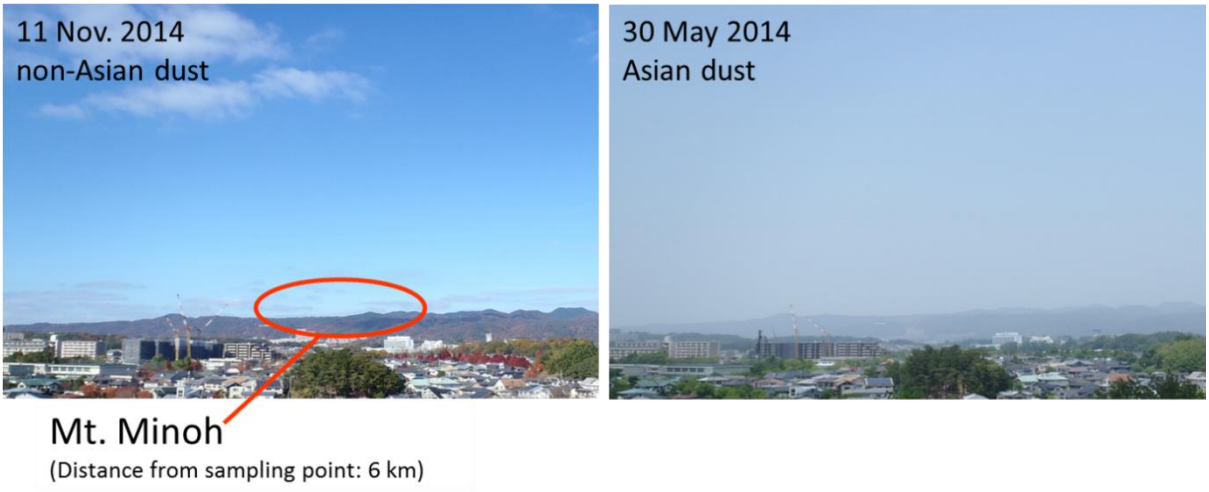

Fig. 1.1. Confirmation of Asian dust events based on visibility from the sampling point (*ca*. 20 m in height).

Table 1.1. Sample descriptions and associated physical characteristics of the atmosphere.

ID	Sampling date	Season	Start	End	Asian dust*	Weather	Temp. (℃)	Relative humidity (%)	Wind speed (m s^{-1})	Wind Direction
1	20130530	Spring	10:00	13:20	-		24.9	N. D.	3	SW
2	20130613	Summer	10:30	13:50	-		18.4	N. D.	3	SW
3	20130627	Summer	9:10	12:30	-	After rainy	26.9	N. D.	3	NW
4	20130722	Summer	10:00	13:20	-		31. 5	N. D.	4	SW
5	20130805	Summer	10:00	13:20	-		33.4	N. D.	3	SW
6	20130820	Summer	10:00	13:20	-		34.7	N. D.	3	SSW
7	20130827	Summer	10:00	13:20	-		30.2	46	3	NW
8	20130903	Fall	10:00	13:20	-		27.5	72	3	ENE
9	20130911	Fall	10:00	13:20	-		30.1	52	2	SSW
10	20130914	Fall	10:20	13:40	-		30.9	59	6	E
11	20130918	Fall	10:00	13:20	-		27.5	50	2	NE
12	20131002	Fall	10:00	13:20	-		25.4	56	2	NW
13	20131017	Fall	10:00	13:20	-	After rainy	19.2	39	6	N
14	20131105	Fall	10:40	14:00	-		18.1	36	3	N
15	20131118	Fall	10:10	13:30	-	After rainy	12.7	31	5	WSW
16	20131203	Winter	10:00	13:20	-		11.9	42	3	SW
17	20140129	Winter	15:30	19:00	-		9.7	24	1	SE
18	20140331	Spring	9:40	13:00	-	After rainy	15.6	23	5	N
19	20140423	Spring	8:40	12:00	-		19.4	31	3	SW
20	20140516	Spring	11:50	15:10	-	After rainy	22.6	36	4	SSW
21	20140517	Spring	12:00	15:20	-		22.2	26	3	NW

22	20140608	Summer	10:40	14:00	-	After rainy	27.3	54	3	SSW
23	20141111	Fall	10:00	13:20	-		17.4	N. D.	1	ESE
24	20141127	Fall	11:00	14:20	-	After rainy	17.0	N. D.	5	N
25	20141217	Winter	10:00	13:20	-	After rainy	3.5	29	6	W
26	20141224	Winter	10:00	13:20	-		8.7	47	2	SW
27	20150114	Winter	10:00	13:20	-		6.5	44	2	ENE
28	20150128	Winter	10:10	13:30	-		5.6	29	6	NNW
29	20150212	Winter	10:00	13:20	-		7.0	47	3	WSW
30	20150227	Winter	10:00	13:20	-	After rainy	8.2	29	5	N
31	20150311	Spring	10:00	13:20	-	After rainy	5.8	30	5	WSW
32	20150416	Spring	11:00	14:20	-	After rainy	18.9	42	4	SW
33	20150417	Spring	10:10	13:30	-		17.2	31	2	N
34	20150423	Spring	11:10	14:30	-		20.7	27	3	S
35	20140520	Spring	10:00	13:20	+		27.2	35	2	N
36	20140527	Spring	11:40	15:10	+		24.1	44	2	S
37	20140528	Spring	10:20	13:40	+		22.4	32	2	SW
38	20140529	Spring	13:10	16:30	+		25.9	30	2	S
39	20140530	Spring	11:40	15:00	+		26.6	35	3	SW
40	20140531	Spring	12:00	15:20	+		28.1	45	3	S
41	20140601	Summer	10:30	13:50	+		28.3	44	3	SW
42	20140602	Summer	10:30	13:50	+		30.0	39	4	SW
43	20140603	Summer	10:30	13:50	+		29.4	41	4	SW
44	20150418	Spring	10:45	15:05	+		19.3	24	3	WSW

* Determined by information obtained from the Japan Meteorological Agency, LIDAR, and visibility at the sampling site.

1.2.3 Estimation of bacterial abundance

To determine bacterial abundance, 16S rRNA gene was quantified by real-time PCR using a LightCycler (Roche Diagnostics, Mannheim, Germany). Real-time PCR was performed with eubacterial primer sets as described by Yamaguchi *et al* (6). A total of 1×10^1 to 1×10^7 copies per reaction of PCR products of *Escherichia coli* W3110 were used as the DNA template to generate a standard curve for quantification of the 16S rRNA gene. The copy number of the 16S rRNA gene differed among bacterial species; therefore, bacterial abundance was calibrated based on the results of bacterial community composition analysis at the phylum level.

1.2.4 Analysis of bacterial community composition

Two-step PCR was conducted to amplify the 16S rRNA gene for amplicon sequencing (21). Using this approach, tags and adapters were added during a second round of PCR amplification. Second round of PCR amplification was performed with 968F (AACGCGAAGAACCTTAC) and 1401R (CGGTGTGTACAAGACCC) sets as described by Ichijo *et al* (22). Amplicon sequencing using an Ion PGM system (Thermo Fisher Scientific KK, Yokohama, Japan) was carried out at the Center for Medical Research and Education, Osaka University (Osaka, Japan). Raw sequence data of the obtained amplicons were screened, trimmed, and filtered using the default settings of QIIME pipeline version 1.9.1 (http://qiime.org/), resulting in over 125,000 sequences across all samples (3,200 sequences per sample, on average). Total operational taxonomic units (OTUs), which were defined at the 97% nucleotide-sequence identity level using the UCLUST function of the QIIME software (23), were identified in all sequences, with about 1,500 OTUs per sample on average being recovered. Beta diversity measures were also calculated. Differences in community composition of each sample were assessed graphically using the ordination method of non-metric multidimensional scaling (MDS) calculated based on the Euclidean distance by PASW statistics 18. Bacterial community composition was finally represented following calibration by copy number of the 16S rRNA gene of each phylum.

1.2.5 Nucleotide sequence accession numbers

The sequences obtained from amplicon sequencing were deposited in the DNA Data Bank of Japan Sequence Read Archive under accession number DRA004472.

1.3 Results

1.3.1 Variations in bacterial abundance on non-Asian dust days and comparison with bacterial abundance on Asian dust days.

Particle size distribution of aerosols in the outdoor environments was measured (Fig. 1.2). The number of particles in the outdoor environment was changed by 10 fold. During Asian dust

events, particle number was generally elevated, and comparatively large particles were dynamically elevated (P value; > 0.3 μm [0.0255], > 0.5 μm [0.0069], > 0.7 μm [0.0011], > 1.0 μm [0.0003], > 2.0 μm [0.0002], > 5.0 μm [0.0028]).

The results revealed a correlation between bacterial abundance and particle size distribution, with particle sizes larger than 1.0 μm showing a greater correlation (Fig. 1.3).

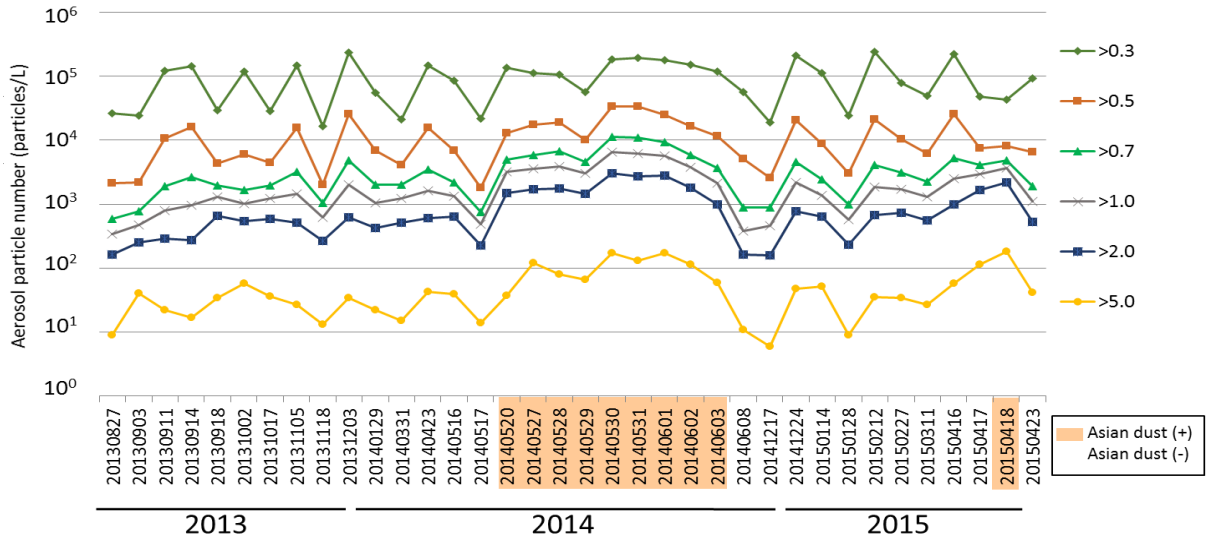

Fig. 1.2. Particle size distribution of aerosols in outdoor environments.

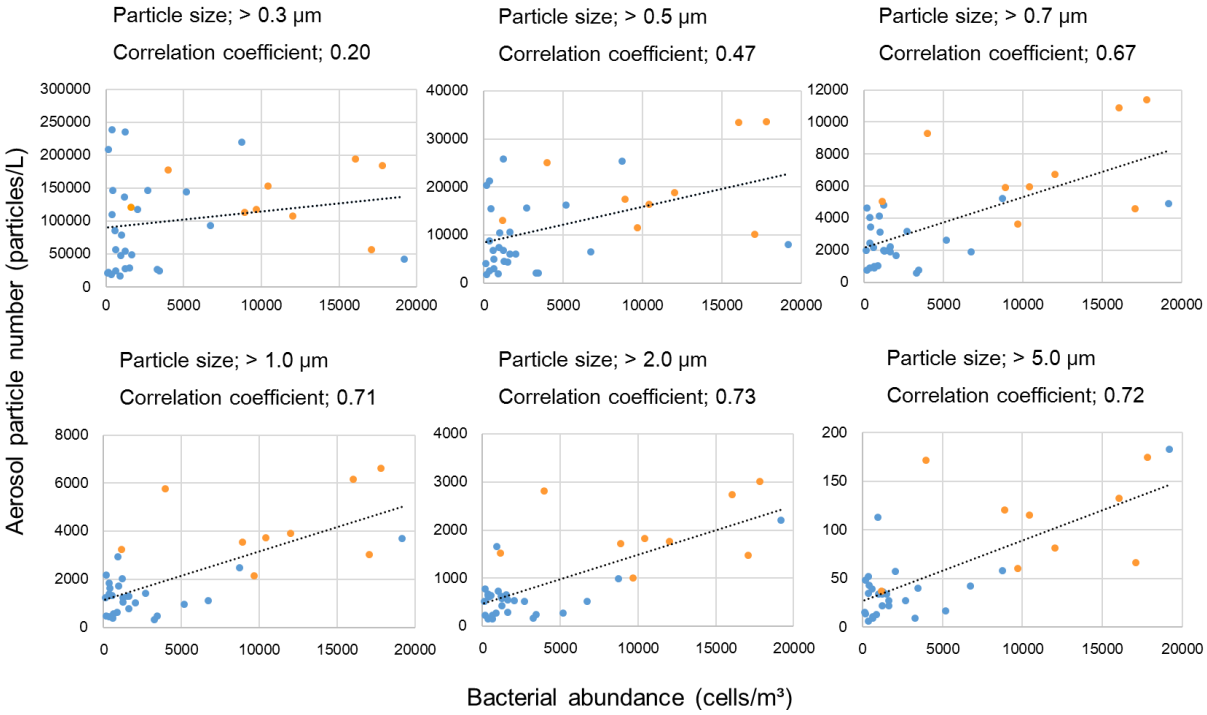

Fig. 1.3. Correlation of bacterial abundance with particle size distribution. The bacterial abundance was determined by quantitative PCR targeting the 16S rRNA gene (V6-V8). Blue and yellow indicate non-Asian and Asian dust samples, respectively.

Accordingly, we compared bacterial abundance and number of particles larger than 1.0 μm during different seasons, rainfall events, and Asian dust occurrences (Fig. 1.4). The levels of particles larger than 1.0 μm fluctuated between 3×10^2 and 3×10^3 L^{-1}, regardless of season. On Asian dust days, particle levels ranged from 2×10^3 to 7×10^3 L^{-1}. Particle numbers on Asian dust days were higher than those on non-Asian dust days, and their fluctuation was more stable on Asian dust days.

Bacterial abundance in outdoor environments varied with variations in particle number (Fig. 1.4; 1×10^2–1×10^4 cells m^{-3}), and bacterial abundance was not influenced by rainfall in this study.

However, bacterial abundance generally increased as the number of particles (> 1.0 μm) increased, without response to seasonal variations or occurrence of Asian dust (correlation coefficient; $r > 0.7$). Bacterial abundance on Asian dust days was generally greater than

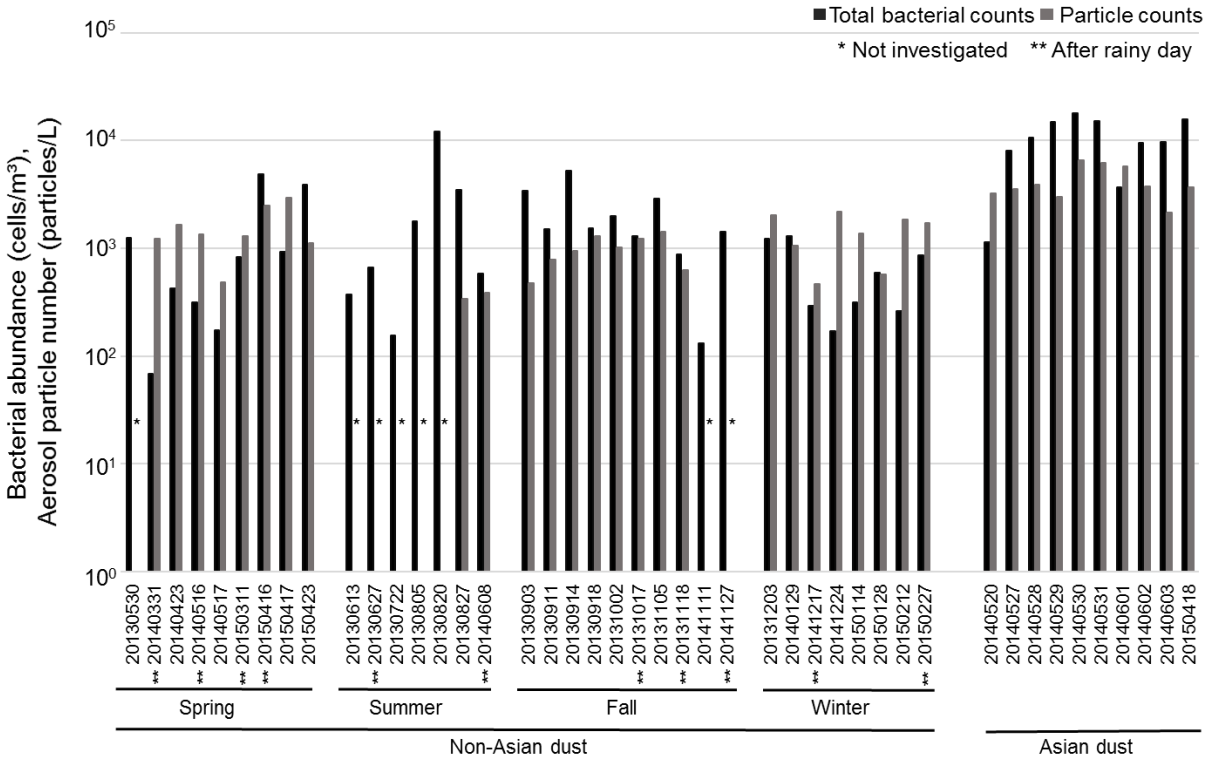

Fig. 1.4. Total bacterial abundance (black bar) determined by quantitative PCR targeting the 16S rRNA gene and total aerosol particle numbers (particle size; > 1.0 μm [gray bar]) determined by particle counter. Samples were collected during each of the four seasons, including after rainy days and Asian dust days.

10^4 cells m^{-3}. The average bacterial abundance on Asian dust days ([1±0.6] × 10^4 cells m^{-3}), increased by approximately 5 times relative to non-Asian dust days ([2±3] × 10^3 cells m^{-3}).

However, bacterial abundance fluctuated from 10^2 to 10^4 cells m^{-3} on non-Asian dust days and changed dynamically relative to Asian dust days, with bacterial abundance reaching that of Asian dust days on several occasions (20 August, 27 August, 3 September, and 14 September, 2013; 16 April and 23 April, 2015).

The ratio of bacterial abundance to number of particles (> 1.0 μm) was comparatively higher in summer and fall (0.56% and 0.28%, respectively). However, this ratio was lower in winter than in other seasons (0.15% in spring, 0.06% in winter). On Asian dust days, the ratio of bacterial abundance to particle number was 0.30% and stable relative to non-Asian dust days.

1.3.2 Variations in bacterial community composition on non-Asian dust days and comparison with bacterial community composition on Asian dust days.

To investigate the bacterial effects of Asian dust, we also analyzed bacterial community composition with variations in environmental conditions on non-Asian dust days. Airborne bacterial community composition in outdoor environments has been reported to change in response to variations in environment factors (15-19). In this study, the airborne bacterial community composition was determined using 16S rRNA gene targeted ion PGM sequencing in conjunction with a two-step PCR method. Two-step PCR has advantages such as increased reproducibility and recovery of higher genetic diversity during amplicon sequencing (22,24).

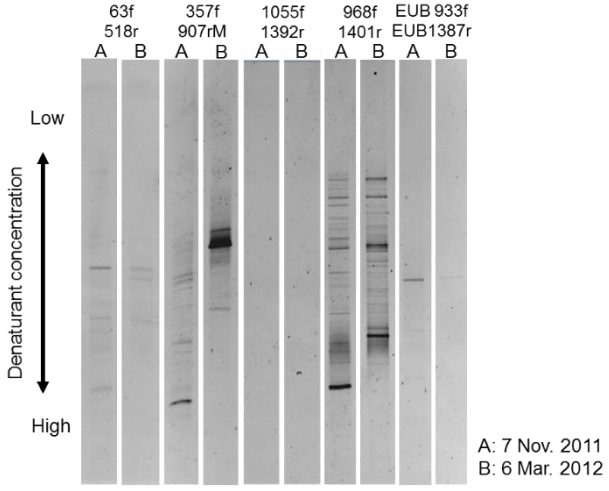

Fig. 1.5. Selection of proper primers using PCR-DGGE. Aerosol samples were collected outdoors using a high-volume air sampler.

In the two-step PCR method, we used the 968f–1401r primer (V6-V8) set because

it produced the highest diversity in a preliminary study using PCR-DGGE to select the proper primer (Fig. 1.5).

To analyze similarities in the bacterial community composition of each sample, amplicon sequencing data of bioaerosol samples were processed using the QIIME software, and the results were indicated using MDS (Fig. 1.6). The results revealed that bacterial community composition in the outdoor environment was rather stable, despite changes in season, and samples were generally not affected by variations in environmental factors. However, the bacterial community compositions of samples collected on 5 August 2013 and 11 November 2014 differed from others. In addition, bacterial community composition did not differ significantly on Asian dust days and non-Asian dust days.

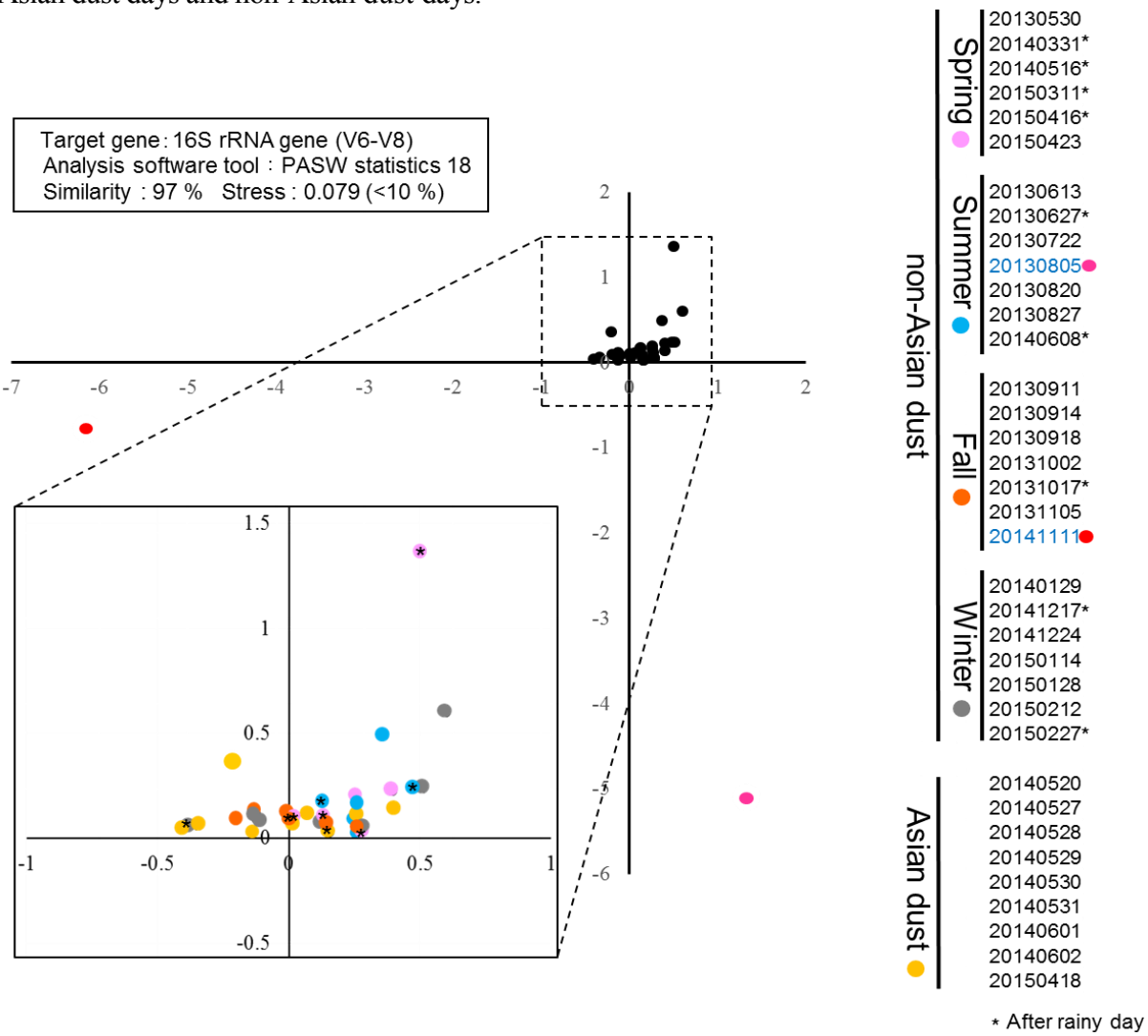

Fig. 1.6. MDS analysis of bacterial 16S rRNA genes obtained from aerosol in outdoor environments.

Comparative analysis of the bacterial community composition at the phylum and class level revealed that environmental factors such as season and rainfall had no effect on the predominant bacterial phylum and class (Fig. 1.7). The predominant phyla and classes on non-Asian dust days were *Acidobacteria* (25±19%), *Actinobacteria* (17±9%), *Bacilli* (15±16%), *Cyanobacteria* (8±7%), *Alphaproteobacteria* (5±4%), *Gammaproteobacteria* (5±9%), *Betaproteobacteria* (4±2%), *Clostridia* (3±2%), and *Deinococci* (2±3%). No specific phylum or class accounted for more than half of the total bacterial community. Variations in the bacterial community composition between seasons did not exceed more than double the original concentration. On Asian dust days, the bacterial community composition was similar to that on non-Asian dust days, with dominant members including *Actinobacteria* (25±9%), *Cyanobacteria* (15±8%), *Acidobacteria* (13±6%), *Bacilli* (11±7%), *Gammaproteobacteria* (7±4%), *Betaproteobacteria* (6±2%), *Alphaproteobacteria* (4±2%), *Deinococci* (3±2%), and *Clostridia* (2±1%). Changes in the outdoor airborne bacterial community composition in

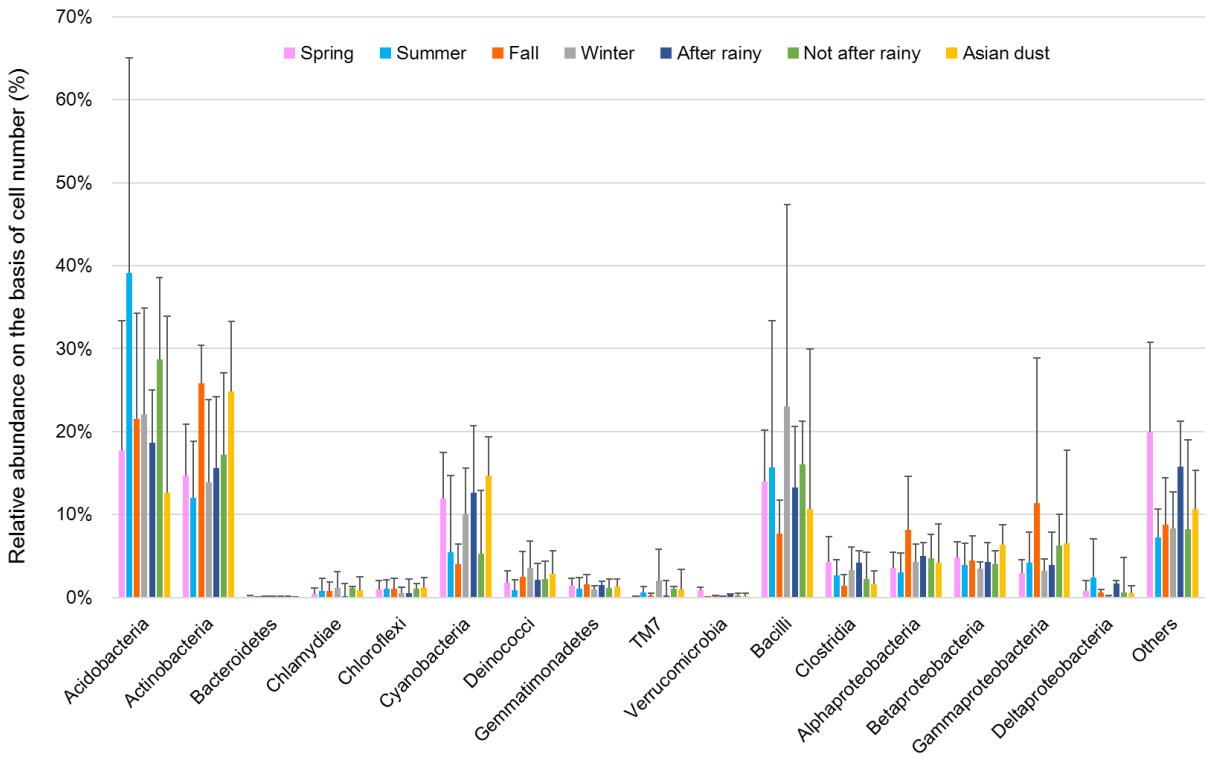

Fig. 1.7. The relative abundance of each common phyla and class in outdoor environment samples (classified in each seasons, weather and Asian dust events).

response to Asian dust did not exceed those observed on non-Asian dust days.

On 5 August, 2013, the bacterial community composition differed from that observed on other sampling dates, with *Acidobacteria* being the dominant member (78%). *Acidobacteria* are generally the dominant phyla in soil habitats (Fig. 1.8) (25). Additionally, on 11 Nov., 2014, *Gammaproteobacteria*, which is known to exist in diverse environments, was dominant (50%).

Bacilli can form spores and withstand severe conditions such as those found in sources of Asian dust (26). There have been many reports of increased levels of *Bacilli* on Asian dust days in downwind regions far from the source regions of Asian dust (16); however, *Bacilli* accounted for more than 50% of the population on several non-Asian dust days in this study (13 June, 2013 [54%], 12 Febraury, 2015 [78%]). *Bacilli* did not increase in response to Asian dust events in this downwind area.

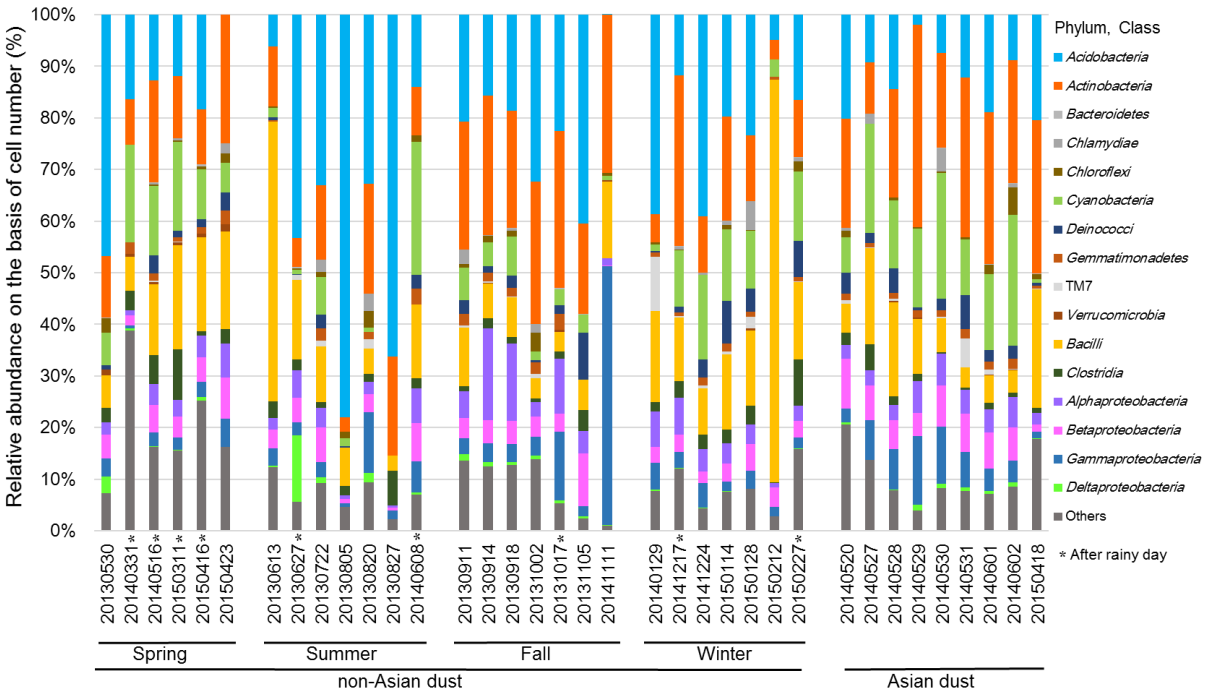

Fig. 1.8. Taxonomic composition for each samples of airborne bacterial communities in outdoor environment. Composition estimates are based on relative abundances of bacterial 16S sequences assigned to different common phyla and class.

1.4 Discussion

In this study, bacterial number and community composition were calibrated based on copy numbers of 16S rRNA of each bacterial phylum. This was conducted because both high copy number bacteria (e.g., *Bacilli*) and low copy number bacteria (e.g., *Acidobacteria*) were present in the collected dust samples. Although bacterial abundance has been reported to change in response to variations in environmental factors (15-19), it was not correlated with any environmental factors (season, temperature, humidity, wind speed, wind direction, rainfall) except particle numbers in the present study. During winter, the ratio of bacterial abundance to particle number was low relative to other seasons. Atmospheric bacterial abundance would be lower in winter because of the response to low temperature (27).

No considerable increase in bacterial abundance was observed on Asian dust days relative to fluctuations in bacterial abundance on non-Asian dust days. Bacterial abundance on aerosols of indoor environments usually ranges from 10^5 to 10^6 cells m^{-3} (28) and bacterial abundance in outdoor environments determined in this study was 10–100 times lower than those in indoor environments.

The results on non-Asian dust days appeared to be correlated with the environmental characteristics of the sampling location. Specifically, we monitored bioaerosols in environments in which temperatures are suitable to the growth of general bacteria (from 4°C to 34°C; average 21±9°C). It has been reported that bacterial abundance and community composition changed significantly in response to season in specific places (e.g., coastal sites [15] high-elevation sites [16]). However, none of our sampling points were located in places such as these. Variations in atmospheric bacterial community composition in outdoor environments impacted by Asian dust occurrence were more stable than those observed on non-Asian dust days.

1.5 Conclusion

Seasonal variations in bacterial abundance of non-Asian dust days were not observed. Bacterial abundance of individual samples collected on non-Asian dust days changed dynamically relative to Asian dust days, with bacterial abundance occasionally reaching those of Asian dust days. The bacterial community composition on non-Asian dust days was rather stable seasonally, and did not differ from that on Asian dust days. Accordingly, these findings indicate that bacterial effects on humans and ecosystems in distant downwind areas impacted by Asian dust may be lower than those of general changes in the natural environment. However, more severe occurrences of Asian dust in areas closer to the dust source may result in microbes in the dust having a greater impact on the indigenous bacterial community. The amount of Asian dust fallout is estimated to be 180 g m^{-2} $year^{-1}$ in Beijing, China (500–2,500 km from the dust source region) (29) and 0.005–0.05 g m^{-2} $year^{-1}$ in Osaka, Japan (3,000–5,000 km from the dust source region) (30). Accordingly, studies of the bacterial community in downwind areas closer to the source are warranted to better assess the impacts of aeolian dust on public health and ecosystems.

Chapter 2

Effects of Asian dust events on atmospheric bacterial communities at different distances downwind of the source region

2.1 Introduction

In the Chapter 1, we suggested that during transportation from the dust source area (the Gobi Desert) to Osaka, Japan (3,200 km from the Gobi Desert), bacterial communities may be affected more by ground environments along the transfer route and local environments than by the bacterial community in the dust itself (31). However, because Asian dust is lifted by updraft and transported by air current, its effect should depend on distance from the dust source region. The Asian dust fallout is estimated as 180 g m^{-2} $year^{-1}$ in Beijing, China (500–2,500 km from the dust source regions) (29) and 0.005–0.05 g m^{-2} $year^{-1}$ in Osaka (3,000– 5,000 km from the dust source regions) (30). The transport of Aeolian dust is influenced by climatic conditions such as air pressure, wind direction and wind velocity, which depend on the ground conditions; moreover, larger dust particles are more difficult to transport over long distances. A previous report confirmed that 55% of the microbial cells detected on Asian dust particles were attached to larger particles (>5 μm), while 7% of them resided on smaller particles (1–2 μm) (31). For this reason, the microbial effect on downwind environments may depend on both the scale of the aeolian dust and the distance from the dust source regions. However, a microbiological comparative analysis of Asian dust events at varying scales and source distances has not been reported.

Therefore, the present study assesses the effects of Asian dust events on atmospheric bacterial communities at different downwind distances from the source regions. To this end, we monitored the bacterial abundance and community composition on outdoor aerosol samples

collected in Beijing during the 2015 Asian dust season (from April to June). We then compared the variations of bacterial abundances and community compositions in Asian dusts collected in Beijing and Osaka (which are close to and distant from the Asian dust source regions, respectively). Airborne bacterial abundances and community structures were determined by 16S rRNA gene-targeted quantitative PCR and amplicon sequencing (32)

2.2 Materials and Methods

2.2.1 Sample collection

Aerosol samples were collected from a second-floor veranda (height *ca*. 5 m) at the China Agricultural University in Beijing, China (latitude: 40°00'14.9" N, longitude: 116°21'10.8" E) and from the rooftop of a building (height *ca.* 20 m) at Osaka University in Osaka, Japan (latitude: 34°9′1.89″ N, longitude: 135°31′15.61″ E) using a high-volume air sampler (HV500R; SIBATA, Saitama, Japan). Air samples were collected onto 0.6-μm pore-size glass fibre filters at 500 L min^{-1}. During each sampling event (200 min), aerosol particles were captured from 100 m^3 of ambient air. In Beijing, 57 samples were obtained from 14 April to 19 June in 2015; in Osaka, 20 samples were obtained from 30 April 2013 to 23 April 2015 (Table 2.1). The occurrence of atmospheric Asian dust was confirmed and their severities were assessed from the increased mass of the glass fibre filter after sampling and the visibility at the sampling location (Fig. 2.1). And it was also consulted visibility data from http://www.chinaairdaily.com/.

2.2.2 DNA extraction

Aerosol samples collected on the glass filter were pulverized by bead-beating (EZ-Beads; EZ, Tokyo, Japan, 4,800 rpm, 90 sec.). DNA was then extracted and purified as described by Tsai and Olson (20). The extracted DNA was subsequently purified using a Wizard DNA Clean-Up System kit (Promega, Madison, WI, USA) and eluted with 50 μL of TE buffer (10 mM Tris-HCl and 1mM EDTA [pH 8.0]).

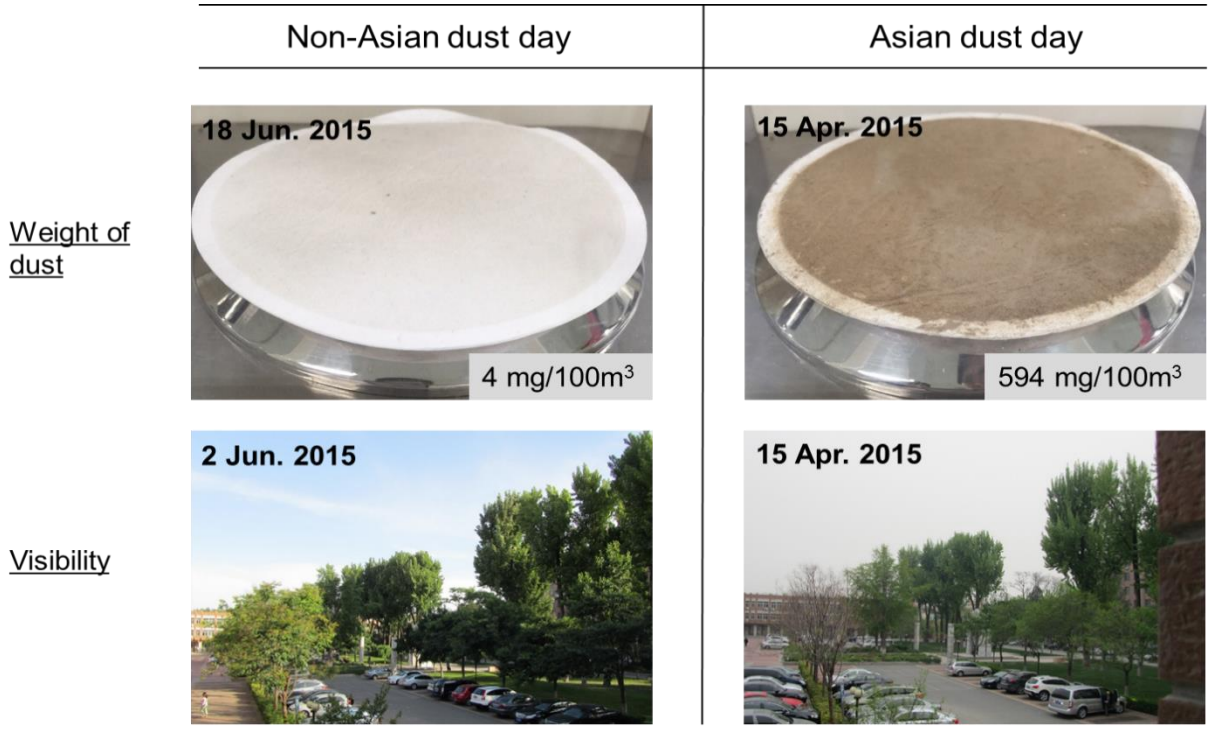

Fig. 2.1. Criteria of Asian dust events in Beijing. Weight of dust and visibility from the sampling point (*ca.* 5 m in height).

Table 2.1. Sample descriptions and associated physical characteristics of the atmosphere.

ID	Sampling date	Location	Asian dust*	Temp. (°C)	Relative humidity (%)	Wind speed (m s^{-1})	Wind direction
1	20150602	Beijing	-	28	29	11	NNE
2	20150606	Beijing	-	24	53	12	N
3	20150607	Beijing	-	22	46	8	WNW
4	20150608	Beijing	-	24	41	10	W
5	20150612	Beijing	-	24	39	18	WNW
6	20150618	Beijing	-	26	37	9	NNW
7	20150619	Beijing	-	22	67	10	ESE
8	20150414	Beijing	+	15	45	7	S
9	20150415	Beijing	+	16	43	10	NNW
10	20150416	Beijing	+	16	13	28	SW
11	20150417	Beijing	+	16	33	11	S
12	20150418	Beijing	+	18	52	5	SE
13	20150419	Beijing	+	16	43	11	SSW
14	20150420	Beijing	+	14	47	9	SSW
15	20150421	Beijing	+	18	37	11	N
16	20150422	Beijing	+	16	36	10	S
17	20150423	Beijing	+	20	34	9	N

18	20150424	Beijing	+	17	39	8	S
19	20150426	Beijing	+	22	48	11	SSW
20	20150427	Beijing	+	22	58	8	WSW
21	20150428	Beijing	+	21	59	8	SSE
22	20150429	Beijing	+	20	64	7	S
23	20150430	Beijing	+	23	57	8	SE
24	20150502	Beijing	+	18	68	7	S
25	20150503	Beijing	+	19	30	15	N
26	20150504	Beijing	+	16	18	17	SSW
27	20150505	Beijing	+	20	31	9	N
28	20150506	Beijing	+	18	17	12	SSE
29	20150508	Beijing	+	18	45	8	SE
30	20150509	Beijing	+	14	59	8	ENE
31	20150511	Beijing	+	14	59	11	NNW
32	20150512	Beijing	+	20	43	14	E
33	20150513	Beijing	+	21	51	7	NW
34	20150514	Beijing	+	22	26	22	SSW
35	20150515	Beijing	+	22	32	10	SSW
36	20150516	Beijing	+	20	43	8	SSW
37	20150518	Beijing	+	23	34	15	N
38	20150519	Beijing	+	20	19	17	N
39	20150520	Beijing	+	21	26	14	SW
40	20150521	Beijing	+	22	37	11	SSW
41	20150522	Beijing	+	21	43	7	SSW
42	20150524	Beijing	+	26	46	8	SSW
43	20150525	Beijing	+	26	43	8	SSW
44	20150526	Beijing	+	28	42	11	S
45	20150527	Beijing	+	26	49	11	SE
46	20150528	Beijing	+	26	52	10	S
47	20150529	Beijing	+	26	59	8	SE
48	20150530	Beijing	+	23	54	8	SSE
49	20150531	Beijing	+	26	47	10	S
50	20150601	Beijing	+	28	54	7	SSE
51	20150603	Beijing	+	24	35	9	SW
52	20150604	Beijing	+	22	67	6	SE
53	20150605	Beijing	+	24	64	6	NNE
54	20150609	Beijing	+	24	50	8	ESE
55	20150610	Beijing	+	21	81	9	ENE
56	20150615	Beijing	+	26	54	7	SSW
57	20150616	Beijing	+	28	56	7	NNW
58	20130530	Osaka	-	25	N.D.	3	SW
59	20130613	Osaka	-	18	N.D.	3	SW
60	20130627	Osaka	-	27	N.D.	3	NW

61	20140423	Osaka	-	19	31	3	SW
62	20140516	Osaka	-	23	36	4	SSW
63	20140517	Osaka	-	22	26	3	NW
64	20140608	Osaka	-	27	54	3	SSW
65	20150416	Osaka	-	19	42	4	SW
66	20150417	Osaka	-	17	31	2	N
67	20150423	Osaka	-	21	27	3	S
68	20140520	Osaka	+	27	35	2	N
69	20140527	Osaka	+	24	44	2	S
70	20140528	Osaka	+	22	32	2	SW
71	20140529	Osaka	+	26	30	2	S
72	20140530	Osaka	+	27	35	3	SW
73	20140531	Osaka	+	28	45	3	S
74	20140601	Osaka	+	28	44	3	SW
75	20140602	Osaka	+	30	39	4	SW
76	20140603	Osaka	+	29	41	4	SW
77	20150418	Osaka	+	19	24	3	WSW

* Determined by weighing the sampled aeolian dust and assessing the visibility in Beijing, and by information obtained from the Japan Meteorological Agency, LIDAR data, and visibility assessments in Osaka.

2.2.3 Estimation of bacterial abundance

To determine the bacterial abundance, 16S rRNA gene was quantified by real-time PCR using a LightCycler (Roche Diagnostics, Mannheim, Germany). Real-time PCR was performed with eubacterial primer sets as described by Yamaguchi *et al* (6). To generate the standard curve for quantifying the 16S rRNA gene, we formed 1×10^1 to 1×10^7 copies per reaction of PCR products of *E. coli* W3110 as the DNA template. As the copy number of the 16S rRNA gene differed among the bacterial phyla, we calibrated the bacterial abundance by a phylum-level analysis of the bacterial community composition.

2.2.4 Analysis of bacterial community composition

The 16S rRNA gene was amplified for pyrosequencing by two-step PCR (21). Two-step PCR increases the reproducibility and recovers higher genetic diversity during amplicon sequencing than one-step PCR (22,24). In this approach, tags and adapters are added in a second round of PCR amplification. Second round of PCR amplification was performed with 968F

(AACGCGAAGAACCTTAC) and 1401R (CGGTGTGTACAAGACCC) sets as described by Ichijo *et al* (22). Amplicons were sequenced using Ion PGM (Thermo Fisher Scientific KK, Yokohama, Japan) at the Center for Medical Research and Education, Osaka University (Osaka, Japan).

The raw sequence data of the obtained amplicons were screened, trimmed, and filtered using the default settings of QIIME pipeline version 1.9.1 (http://qiime.org/). Over 278,000 sequences were obtained across all samples (averaging 5,100 sequences per sample). Total operational taxonomic units (OTUs), defined at the 97% nucleotide-sequence identity level using the UCLUST function of QIIME software (23), were identified in all sequences. On average, approximately 2,800 OTUs per sample were recovered. The community composition differences among the samples were graphically assessed by principal coordinate analysis (PCoA) using the unweighted pair group method. Finally, the bacterial community composition was calibrated by the copy number of the 16S rRNA gene of each phylum and class.

2.2.5 Nucleotide sequence accession numbers

The sequences obtained from the amplicon sequencing were deposited in the DNA Data Bank of Japan Sequence Read Archive under the accession number DRA004472.

2.3 Results

2.3.1 Comparisons of bacterial abundance variation during an Asian dust season in Beijing and Osaka

During the Asian dust event, the bacterial abundance in Beijing's outdoor environment was approximately 40 times higher than on non-Asian dust days (Asian dust days: $[4 \pm 3] \times 10^4$ cells m^{-3}, non-Asian dust days: $[1 \pm 0.5] \times 10^3$ cells m^{-3} ; $P = 0.01$) (Fig. 2.2). The bacterial abundances on non-Asian dust days were not significantly different in Beijing

and Osaka ([1 ± 0.5] × 10^3 cells m^{-3}). The Asian dust event altered the bacterial abundance to a greater extent in Beijing than in Osaka (Beijing; [4 ± 3] × 10^4 cells m^{-3}, Osaka; [1 ± 0.2] × 10^4 cells m^{-3} ; P = 0.04). Although the bacterial abundance on Asian dust days generally exceeded 10^4 cells m^{-3} in both areas, high bacterial concentrations (>2 × 10^4 cells m^{-3}) were confirmed in Beijing on some days (14, 15, 26, 30 April and 5 May 2015), but were not observed in Osaka. The bacterial concentration in Beijing was especially high on 15 April 2015 (10^6 cells m^{-3}), coinciding with the exceptional severity of the Asian dust. On that day, the concentration of the bacteria transported to Beijing was approximately 100 times higher than on other Asian dust days, and 1,000 times higher than on non-Asian dust days.

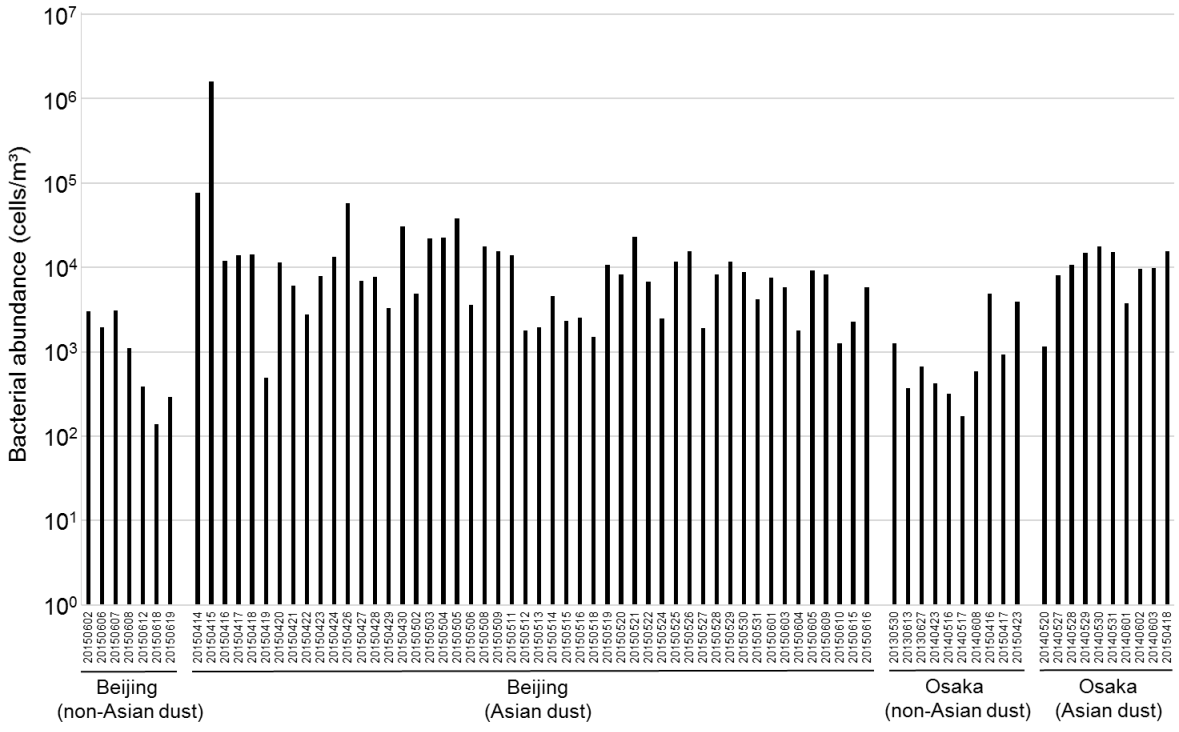

Fig. 2.2. Total bacterial abundance determined by quantitative PCR targeting the 16S rRNA gene. Samples were collected during the 2015 Asian dust season in Beijing and Osaka. Included are non-Asian dust days and Asian dust days.

2.3.2 Comparisons of bacterial community compositions in Beijing and Osaka during the Asian dust season

In Beijing, the transported Asian dust was dominated by *Actinobacteria* (28 ± 1%), *Bacilli* (22 ± 1%), *Acidobacteria* (11 ± 1%), *Clostridia* (6 ± 1%), *Alphaproteobacteria* (6 ± 0.4%) and *Betaproteobacteria* (6 ± 1%) (Fig. 2). On non-Asian dust days, the predominant phyla and classes were *Actinobacteria* (27 ± 5%), *Bacilli* (14 ± 3%), *Alphaproteobacteria* (14 ± 3%), *Betaproteobacteria* (11 ± 2%), *Cyanobacteria* (11 ± 2%) and *Acidobacteria* (7 ± 3%). The *Bacilli* and *Clostridia* levels were increased by Asian dust events in Beijing (*Bacilli*: P = 0.028, *Clostridia*: P = 0.00027). *Cyanobacteria* increased from the end of May, regardless of Asian dust events (before 20 May: 2 ± 0.4%, after 20 May: 9 ± 2%; P = 0.000013) (Fig. 2.3).

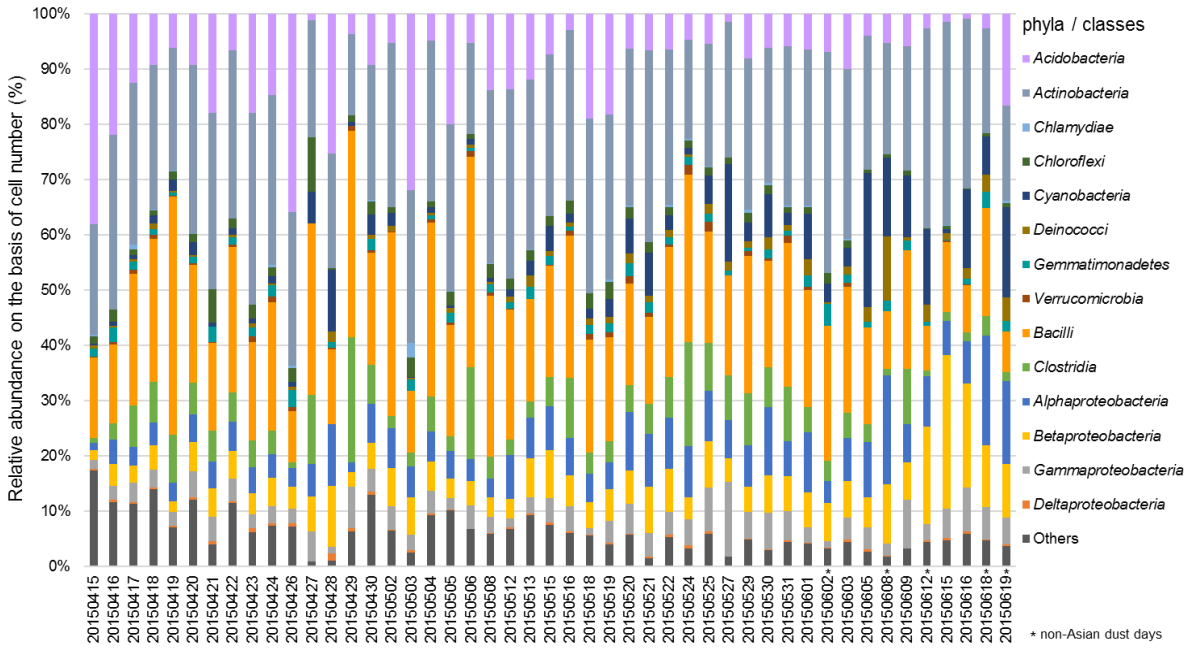

Fig. 2.3. Taxonomic compositions of the outdoor airborne bacterial communities in each sample collected in Beijing. Compositions were estimated from the relative abundances of the bacterial 16S rRNA gene sequences assigned to different common phyla and classes.

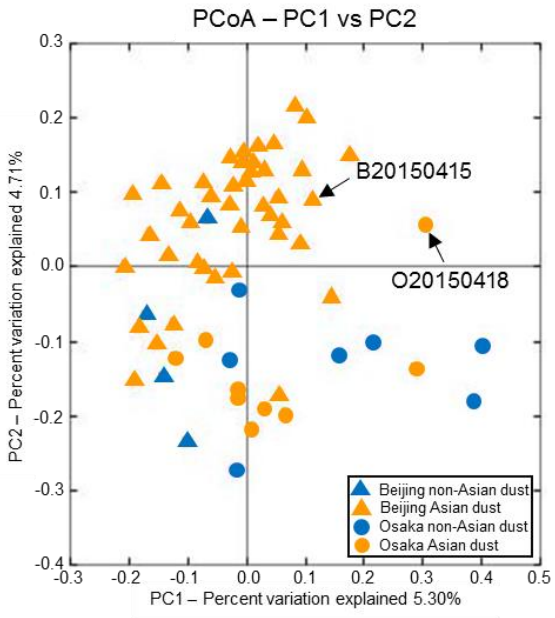

Fig. 2.4. PCoA of bacterial community composition based on the 16S rRNA gene sequences obtained from aerosols collected in outdoor environments. B20150415: Asian dust collected in Beijing, 15 April 2015. O20150418: Asian dust collected in Osaka, 18 April 2015.

To analyse the similarities in the bacterial community compositions of different samples (including those collected in Osaka), we processed the amplicon sequencing data using QIIME software (23), and displayed the results on a PCoA plot (Fig. 2.4). The clustering differed between Asian and non-Asian dust days in Beijing, but not in Osaka. The bacterial community compositions on Asian dust days were more closely related in Beijing than in Osaka. The Asian dust observed in Beijing on 15 April 2015 reached Osaka on 18 April 2015 (Fig. 2.5). The bacterial community compositions of these two Asian dust samples were more similar than the 15 April sample collected in Beijing and the samples collected in Osaka on days excluding 18 April 2015. However, similarity of the community compositions between the 15 April sample collected in Beijing and samples collected in Beijing on days excluding 15 April 2015 was higher than that between the 15 April sample collected in Beijing and the 18 April sample collected in Osaka. During the Asian dust event, the bacterial phyla/classes that dominated more than 10% of the total community in Beijing changed from *Actinobacteria, Cyanobacteria, Bailli, Alphaproteobacteia, Betaproteobacteria* to *Acidobacteria, Actinobacteria* and *Bacilli* (Fig. 2.6). In Osaka, the Asian dust occurrence changed the dominant phyla/classes from *Acidobacteria, Acitinobacteria* and *Bacilli* to *Acidobacteria, Actinobacteria, Cyanobacteria* and *Bacilli*. That is, the Asian dust event altered the diversity of the airborne bacterial community composition more in Beijing than in Osaka. In Beijing, the most affected groups were *Bacilli* (14 ± 3% to 22 ± 1%) and *Clostridia* (from 2 ± 1% to 6 ± 1%).

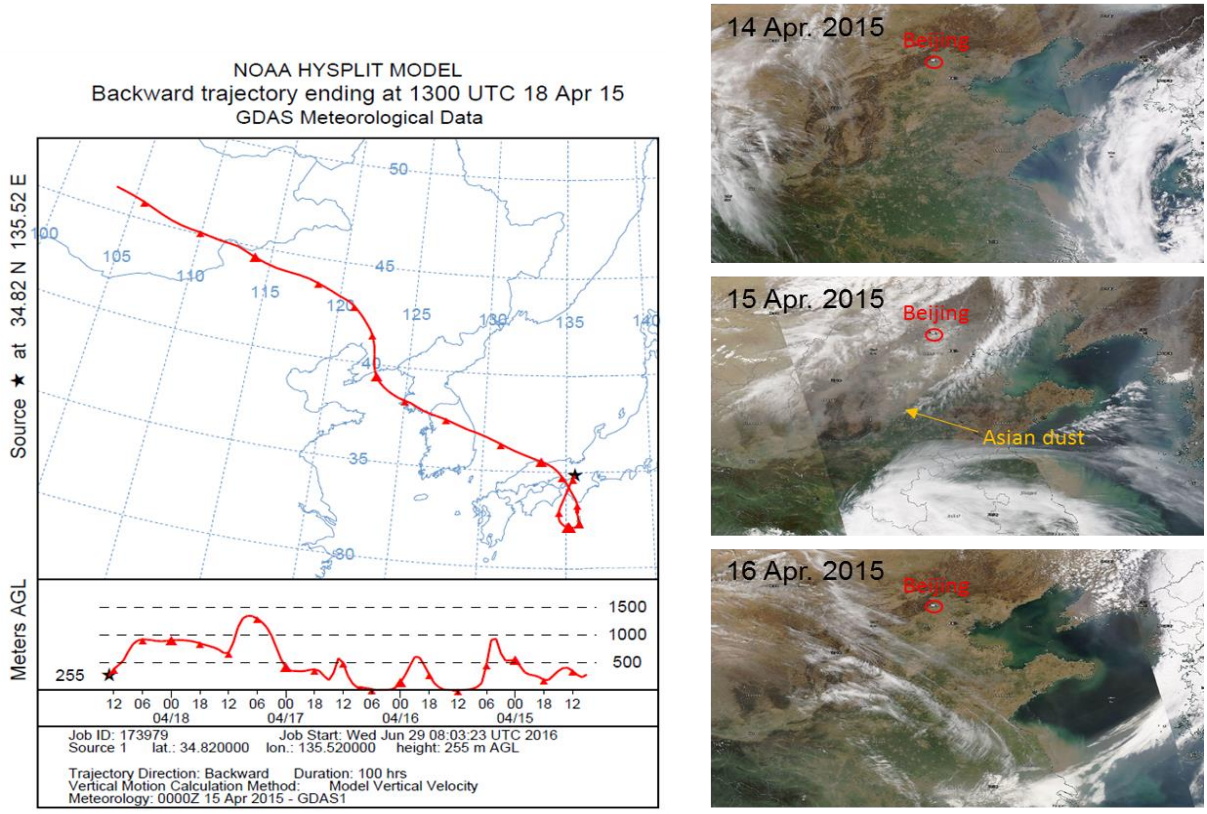

Fig. 2.5. The geographic origin of Asian dust was determined by back trajectory analysis (left; http://ready.arl.noaa.gov/HYSPLIT.php) and was rendered in EOSDIS Worldview (right; https://worldview.earthdata.nasa.gov/).

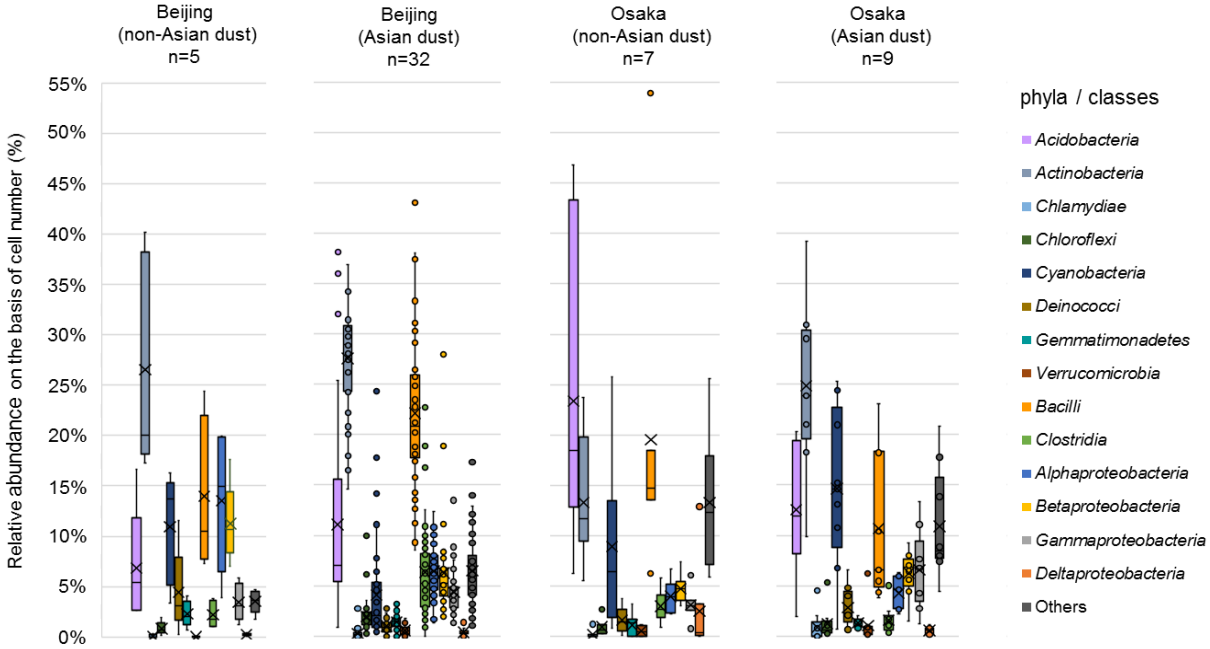

Fig. 2.6. Relative abundances of the common phyla and classes in outdoor airborne samples. Data were classified by their sampling points and Asian dust events.

As mentioned above, the Asian dust observed on 15 April 2015 in Beijing reached Osaka on 18 April 2015 (Fig. 2.5). Therefore, we conducted a comparative analysis of the bacterial community compositions in both areas (Fig. 2.7). The bacterial communities in Beijing and Osaka were dominated by the same groups (*Acidobacteria, Actinobacteria* and *Bacilli*). These phyla/classes comprised over 70% of the whole bacterial community. However, during the transport from Beijing to Osaka, *Acidobacteria* decreased from 38% to 20%, while *Actinobacteria* and *Bacilli* increased from 20% to 30% and from 14% to 23%, respectively. The other phyla/classes were not significantly changed.

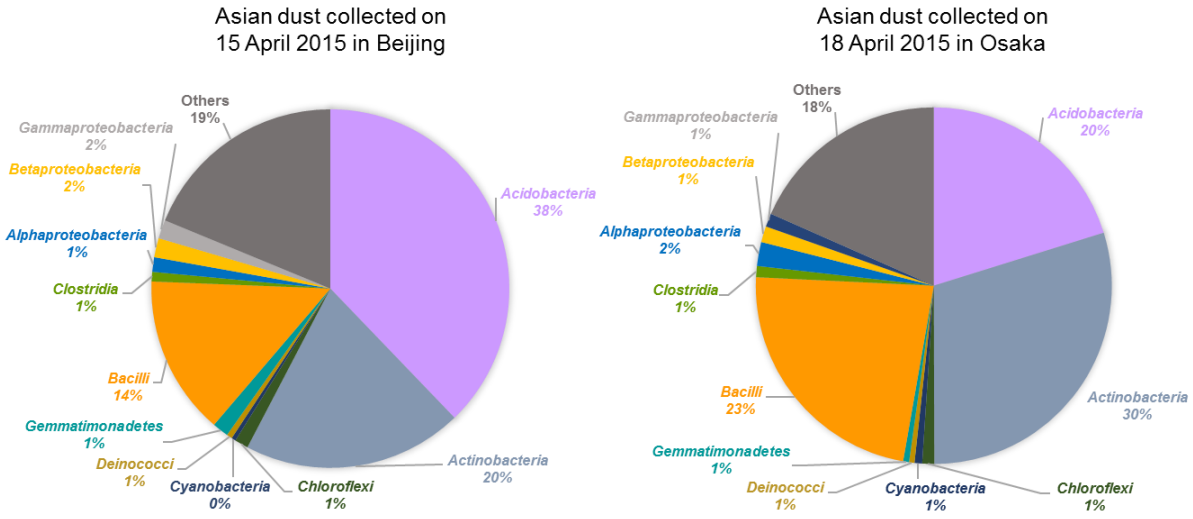

Fig. 2.7. Taxonomic compositions of Asian dust samples collected in Beijing and Osaka during the same Asian dust events (15 and 18 April, 2015). Compositions were estimated from the relative abundances of bacterial 16S rRNA gene sequences assigned to the common phyla and classes.

In addition, when the Asian dust days increased the bacterial abundance to over 10^4 cells m^{-3}, the *Acidobacteria* concentration was higher than on non-Asian dust days (increasing from 7 ± 1% to 16 ± 3%; Fig. 2.8).

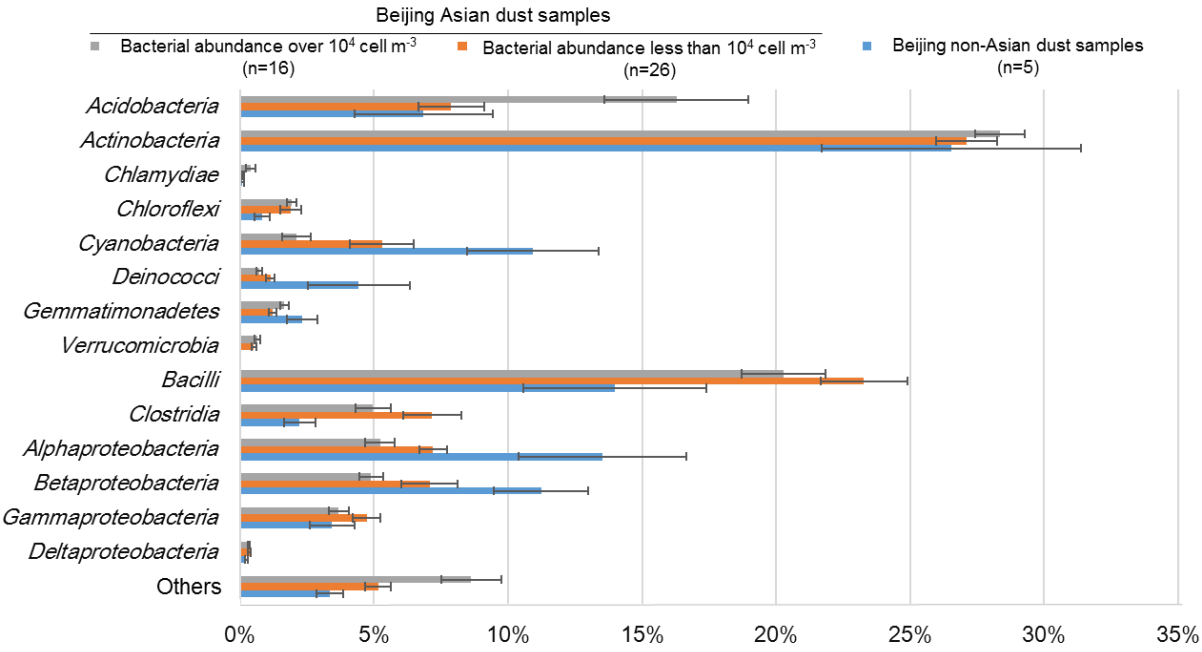

Fig. 2.8. Relative abundances of the common phyla and classes in outdoor airborne samples collected in Beijing. Data were classified by the Asian dust events and their bacterial abundance on Asian dust days.

2.4 Discussion

In this study, bacterial number and community composition were calibrated based on copy number of 16S rRNA gene of each bacterial phylum and class (31). This was conducted because both high copy number bacteria (e.g., *Bacilli*) and low copy number bacteria (e.g., *Acidobacteria*) were present in the collected dust samples.

We collected aerosol samples in Beijing (700 km from the dust source region) and Osaka, Japan (3,200 km from the dust source region) and evaluated the changes in the outdoor airborne bacterial community as the dust travelled downwind to Osaka. This investigation revealed the distance-dependent effects of the Asian dust event on the atmospheric bacterial community. The results confirmed that the bacterial abundance increases near the dust source region, and that aeolian dust alters the bacterial community composition more in close regions than in regions far from the source. In Beijing, the Asian dust increased the levels of spore forming bacteria such as *Bacilli* and *Clostridia*. Many studies have reported similar abundances of *Bacilli* and *Clostridia* in the source region of Asian dust and in downwind regions in China (12,26,33).

Acidobacteria especially dominated the Beijing bacterial community during the Asian dust days increased the bacterial abundance to over 10^4 cells m^{-3}, including severe 15 April 2015 event. *Acidobacteria* is especially distributed in soils (34), and dominates in arid and semi-arid regions (35-37). Meanwhile, during the long-range transportation of Asian dust particles from Beijing to Osaka, the percentage of *Acidobacteria* in the total bacterial community decreased while the *Actinobacteria* and *Bacilli* levels increased. *Actinobacteria* has high tolerance to environmental stresses and inhabits extreme environments such as hypersaline lakes, thermal springs, and arid soils. *Bacilli* survive stressful environments by forming spores. Because most bacteria transported by aeolian dust experience ultraviolet exposure, reduced nutrient availability, desiccation and other stresses, the stress-tolerant groups *Actinobacteria* and *Bacilli* may persist while other phyla/classes diminish.

2.5 Conclusion

This study monitored the bacterial abundance and community composition in outdoor aerosol samples in Beijing, China, which is close to the Asian dust source regions, and compared them with the results obtained in a distant region (Osaka, Japan). The Asian dust collected in Beijing contained $(4 \pm 3) \times 10^4$ bacterial cells m^{-3}, approximately 4 times higher than in Osaka. On 15 April 2015, Beijing experienced severe Asian dust events with a 1000-fold increase in bacterial abundance, relative to non-Asian dust days. Dominant bacterial phyla and classes in Asian dust collected in Beijing were Actinobacteria, Bacilli and Acidobacteria, and the bacterial community composition varied more widely than in Osaka. The bacterial community compositions differed between the Beijing and Osaka dusts, even for the same Asian dust events. Therefore, aerosol bacterial communities nearer the dust source are more affected by aeolian dust than their distant counterparts.

General Conclusion

The present study was conducted to investigate the effects of bacteria transported by Asian dust events on human health and ecosystems using microbiological analyses of outdoor aerosol samples from Osaka, Japan. Samples were collected on both Asian dust and non-Asian dust days from 2013 to 2015. Moreover, to investigate the differences in microbial effects along a downwind trajectory, we also monitored bacterial abundance and community structure in outdoor aerosol samples from Beijing, China, nearer to the Asian dust source regions, during the Asian dust storm season of 2015.

We demonstrated that bacteria transported long distances by Asian dust did not immediately influence the local atmospheric bacterial community in distant downwind areas, such as Osaka (located 3,000–5,000 km from the source region). Our findings suggest that transported bacterial communities may be affected more by ground environments along the transfer route and local environments than by the bacterial community in the dust itself in distant downwind areas. In contrast, in Beijing (located 500–2,500 km from the source region), Asian dust increased bacterial abundance and the levels of specific bacteria, such as Acidobacteria, Bacilli, and Clostridia. In particular, a severe Asian dust event in Beijing on 15 April 2015 increased the bacterial abundance 1,000-fold compared with non-Asian dust days. The same event increased the bacterial abundance in Osaka only 10-fold, compared with non-Asian dust days. During this long-range transport of Asian dust particles from Beijing to Osaka, the percentage of Acidobacteria in the bacterial community decreased, while both Actinobacteria and Bacilli levels increased. Because most bacteria transported by aeolian dust are influenced by UV exposure, reduced nutrient availability, and other stresses, this suggests that Bacilli and Actinobacteria have higher viability compared with bacteria from other phyla/classes. These results indicate that bacteria attached to Asian dust particles are clearly affected by

environments experienced along the transfer route. Thus, the effects of bacteria transported by Asian dust events on humans and ecosystems probably depend on both the distance from the dust source region and the scale of the dust event.

Acknowledgements

To begin with, I would like to express my gratitude to all those who made this dissertation possible. In particular, I would like to sincerely thank my research advisor, Professor emeritus Masao Nasu, for giving me the opportunity to undertake my PhD studies in his laboratory. I valued his support and academic guidance throughout my doctoral studies.

I am deeply grateful to Dr. Nobuyasu Yamaguchi at the Osaka Prefectural Institute of Public Health for his strong support of my research work, publications, and at my thesis defense. It was a great pleasure to carry out my work under his mentoring.

I would also like to thank Assistant Professor Tomoaki Ichijo at Osaka University for his support and academic guidance, as well as Dr. Takashi Baba at Tottori University for invaluable advice and encouragement throughout my degree. In addition, I would like to thank Toshiaki Hayashi for his technical assistance and support of my research work.

Furthermore, I am deeply grateful to Dr. Gun-hee Lee, Dr. Min-su kim, Dr. Se-min Park, Dr. Min-young Kim, Dr. Dan-bee Ha, Young-woo Jo, Ayaka Kawabata, and Sachiko Teramoto for their warm encouragement and friendly help. I was glad to have meet them and they will not be forgotten. I want to thank all members of the Environmental Science and Microbiology Laboratory for their support and cooperation during my studies.

Lastly, I thank my family for their endless support and encouragement.

Osaka, Japan 2017

Jonguk Park

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