Phylogenetic Analysis of Atmospheric Microbial Communities Transported by Asian Dust (Kosa) Events

Phylogenetic Analysis of Atmospheric Microbial Communities Transported by Asian Dust (Kosa) Events

黄砂によって風送される大気微生物群集の分子系統解析に関する研究

being a Thesis submitted for the Degree of
Doctor of Philosophy

by:
Findya Puspitasari
1223132009

GRADUATE SCHOOL OF NATURAL SCIENCE & TECHNOLOGY
KANAZAWA UNIVERSITY
2015

Dissertation

Phylogenetic Analysis of Atmospheric Microbial Communities Transported by Asian Dust (Kosa) Events

Graduate School of
Natural Science & Technology
Kanazawa University

Division of Material Sciences

Student ID No. 1223132009
Name : Findya Puspitasari
Chief advisor : Assoc. Prof. Maki Teruya
July 2015

Abstract

Airborne microorganisms (bioaerosol) from the Chinese desert region, which are released into the atmosphere, disperse by the Asian dust event and affect ecosystems, human life, atmospheric processes and ice-cloud formation in downwind areas. However, the dynamics of airborne bacterial abundance and compositions have rarely been investigated in Asian dust source region and downwind areas during dust events. In the first segment of the study, air samplings were sequentially performed in the Asian dust arrival area (Kanazawa city, Japan) during a dust event. The airborne bacterial communities in dusty atmosphere significantly differed from those in the non-dust period and dominated by terrestrial bacteria such as *Bacillus* species.

Next the bacterial communities in air samples and sand samples of the Asian dust source region (Taklamakan desert) were analyzed using a clone library technique. Air samples were mainly composed of the members of *Firmicutes* and *Proteobacteria* and the proportion of *Proteobacteria* sequences decreased during a dust event, whereas that of *Firmicutes* clones increased correspondingly. *Bacillus* species was predominant in both sand and air samples. These results suggested that the transports of bioaerosols from dune sand to atmosphere.

Finally, for more comprehensive understanding in vertical mixture of airborne microbial to high altitudes over Asian dust source, the air samples collected at altitudes of 800 m and 10 m over Taklamakan desert were analyzed using pyrosequencing targeting bacterial 16S rDNA and fungal Internal Transcribed Spacer (ITS) regions. High diverse of microorganisms were detected

from the samples of 10 m. while some populations of bacteria (*Bacilli* and *Gamma-proteobacteria*) and fungi (*Cladosporium* species) were found to suspend at the high altitude of 800 m. Dust events over Taklamakan desert are thought to carry fungal populations as well as bacterial populations. The dominated levels of fungal populations were higher than those of bacterial populations, suggesting that the most fungal cells would be hardly transported to higher altitudes.

The wind conditions over desert area would contribute to vertical mixture of some microbial populations from dune sand to atmosphere and the bacterial population that maintain their viabilities increased in atmosphere in downwind areas.

Contents

List of Tables

List of Figures

Chapter 1:

GENERAL INTRODUCTION

1.1 BACKGROUND AND RESEARCH OVERVIEW

The soil particles, which are derived from the deserts of the Asian continent, are frequently transported over the Eastern China Sea or the Yellow Sea to Japan during the spring season (Iwasaka et al. 1983). These Asian dust events are common atmospheric phenomena in Japan, and are known as “Kosa” that literally means yellow sands in Japanese (Iwasaka et al. 1983). Desert winds aerosolize several billion tons of soil derived dust each year, including concentrated seasonal pulses from the Asian desert areas (Uematsu et al. 1983; Duce et al. 1980), causing the damages of human health (Ichinose et al. 2005).

In addition, Kosa events are known to transport airborne microorganisms, thereby supporting the microbial immigration to downwind ecosystems (Maki et al. 2010; Iwasaka et al. 2009). The mineral-dust particles are associated with organic particles commonly called “bioaerosols,” which include viruses, bacteria,

fungi, and pollen as well as plant and animal debris (Jones and Harrison 2004; Jaenicke 2005; Prospero et al. 2005). Possibly the transportation of bioaerosol by Kosa events have a role in transporting pathogens (Kellogg and Griffin 2006; Iwasaka et al. 2009). The Airborne microorganisms carried by dust event contributing to the dispersion of diseases such as Kawasaki diseases in human (Rodo et al. 2011) rust diseases in plant (Brown and Hovmoller 2002), and increase the allergen burden causing an increase incidence in asthma (Ichinose et al. 2005). Mouse assay suggested that Kosa events could transport microbial matters and allergens with the potential to impact the health of downwind populations and ecosystems (Liu et al. 2014).

Some types of airborne microorganisms that can synthesize organic molecules such as mannitol, glucose, and fructose, impact the organic carbon content of cloud water and aerosols (Delort et al. 2010). Microorganisms in the atmosphere are also known to act as ice nuclei (Joly et al. 2013) and cloud condensation nuclei affecting ice-cloud processes (Möhler et al. 2007). Viable bacteria have been identified in super cooled cloud droplets, and their ability to metabolized organic matters in the environment has been confirmed (Sattler et al. 2001; Vaïtilingom et al. 2012). Bioaerosols are also thought to influence atmospheric processes by participating in atmospheric chemical reactions and formation of cloud-nucleating particles (Pratt et al. 2009).

Bacterial community structures in the atmosphere should be investigated for understanding the long-range transport processes of airborne bacteria and their influence on ecosystem dynamics, human health, and climate change in downwind areas. The researches targeting atmospheric bioaerosols has developed

very rapidly since the 21 century (Jones and Harrison 2004; Jaenicke 2005, Prospero et al. 2005; Iwasaka et al. 2009), but accumulated data of microbial communities in the atmosphere is limited to a few studies, which have focused on the microorganisms traveling with the African dusts (Kellogg and Griffin. 2006; Kellogg et al. 2004) and Kosa (Wu et al. 2004; Yeo and Kim 2002). Then the microbial community structures of the atmospheric bioaerosols in the source and arrival areas of Kosa were not clarified.

The atmosphere is a heterogeneous environment; meteorological shifts and dust events considerably alter bacterial abundance and composition in the Asian atmosphere (Hara and Zhang, 2012; Maki et al. 2014). The airborne microorganisms at high altitudes (approximately 3,000 m above sea level) over dust arrival areas were found to change in response to Kosa events that included highly diverse bacterial species (Smith et al. 2012; Maki et al. 2013). The Kosa mineral particles are mainly originated from the Chinese desert areas such as Taklamakan desert, Loess Plateau, and Gobi deserts (Sun et al. 2001; Duce et al. 1980; Iwasaka et al. 1983). The sand dune (Hua et al. 2007; An et al. 2013) and atmosphere (Hua et al. 2007; Maki et al. 2008; Wang et al. 2010) in Chinese desert areas has been demonstrated to include high diverse of bacterial communities. However, the dynamics of airborne bacterial communities in Kosa source and arrival regions have not investigated in detail. The vertical mixture process of bioaerosols over Asian source region is unclear inhibiting the determination of long-range transported microorganisms.

In chapter 2, for elucidating the airborne bacterial dynamics in Kosa downwind area such as Japan, air samples were collected at the ground surface in

Kanazawa city during a long-term of Kosa event, and the changes in bacterial abundance and species compositions were determined using the combination of fluorescence microscopic observation and clone library analysis targeting 16S rRNA genes (16S rDNA). In chapter 3, dune sand samples and air samples of the Kosa source region (Dunhuang city in Taklamakan desert) were collected for analyzing the dynamics of airborne bacteria in Dunhuang city during a dust event and comparing between the bacterial communities of sands and atmosphere depending on clone library analysis. In chapter 4, air samplings using a balloon and a building were performed for collecting air samples at altitudes of 800 m and at 10 m for more comprehensive understanding of airborne microbial communities at high altitudes in Asian-dust source. Microbial community structures in the air samples were analyzed using the combination of pyrosequencing analysis and cloning library technique.

Chapter 2:

AIRBORNE BACTERIAL COMMUNITIES STRUCTURE VARIATION DURING ASIAN DUST (KOSA) EVENT IN A DOWNWIND AREA, KANAZAWA CITY JAPAN

2.1 INTRODUCTION

The dynamic of airborne bacteria in downwind areas during Kosa event need to be elucidated in order to understand the characteristics of bacterial communities that are transported long distances and influences downwind ecosystem and climates. The size and composition of airborne bacterial communities at high altitudes above Kosa dust deposition areas such as Beijing (Li et al. 2010), Osaka (Yamaguchi et al. 2012), Noto Peninsula (Maki et al. 2013), and the North American mountains (Smith et al. 2012) varied significantly depending on the Kosa event studied. Investigations of airborne microbial

dynamics at the ground level in China and Korea indicated that soma bacterial species from *Firmicutes* were predominant during Kosa events (Jeon et al. 2011). However, there are a few reports investigating bacterial dynamics at the ground level in Japan when the dust particles that have passed through China and Korea have traversed the Sea of Japan. Dust particles reaching Japan are reported to change their chemical composition when passing over Chinese industrial area and the Sea of Japan. Thus, the chemical and biological characteristics of dust particles in Japan are expected to differ from the dust particles collected from Korea and China, and, in fact, it was reported that bacterial abundance and viabilities at the ground level in Japan dynamically changed during a Kosa events (Hara and Zhang 2012). However, the diversity and structure dynamics of airborne bacteria have yet to be investigated in a populated area in Japan during a Kosa event.

To investigate the population dynamics of airborne bacteria in Japan during a Kosa event, we collected air samples at the ground surface in Kanazawa city from May 1 to May 7, 2011, during a long-term of Kosa event. We determined the abundance of bioaerosol in the air samples by microscopic observation using a fluorescence staining technique. The composition of the bacterial species in the air samples was analyzed using clone-library analysis targeting bacterial 16S ribosomal RNA genes (16S rDNA).

2.2 MATERIALS AND METHODS

2.2.1 Sampling

Air sampling was performed in Kanazawa city (36.33ºN, 136.39ºE), a

coastal city located on the northern coast of the Hokuriku region on the south area of Japan Sea. In this city aerosol from the continental area arrived directly from the Sea of Japan and aerosol contamination from Japan can be eliminated. Sampling was performed from 7:00 JST on May 1, 2011 to 8:00 JST on May 7, 2011, during dust event (1-4 May, 2011). The sampling system was placed on a 10-m high platform (located at Kanazawa University). Air samples (520 L) were collected using sterilized polycarbonate filters (0.22 μm pore size; Whatman, Tokyo, Japan) with a sterilized filter holder using an air pump. For each sample, two filters were used continuously for 12 h; the filters were changed every 12 h. In total, four air samples were obtained during the sampling period from the morning of May 1 to the morning of May 7, which were labeled sample 1 to sample 12. Of the two filters used to collect each sample, one filter was used to determine the abundance of bioaerosol by microscopic observation, and the other one was stored at –80°C before the extraction of genomic DNA for the analysis of bacterial species composition.

2.2.2 Characteristics and trajectories of air masses

To track the transport pathways of air masses, 72-h backward trajectories were calculated using the National Oceanic and Atmospheric Administration (NOAA) Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model (http://www.arl.noaa.gov/HYSPLIT.php). The position of the backward trajectory start point was used as the air sampling area for this study (36.33ºN, 136.39ºE), from altitudes 10, 1000 and 3000 m above the ground level to estimate the accurate trajectories of air masses in the free troposphere. The depolarization

ratios of particle below 3000 m on May 1 and May 7, 2011 were measured using light detection and ranging (lidar) at Toyama city (http://www-lidar.nies.go.jp/).

Ground surfaces data for the atmospheric and air quality were obtained from the meteorological observatories of the Japan Meteorological Agency in Kanazawa city that located 10 km from sampling site and Wajima city that located 50 km from sampling site. For analyzing air-mass dynamics every one hour environmental data were collected such as precipitation, temperature, relative humidity, wind velocity, dew point temperature, and steam pressure (Table 2.2)

2.2.3. Microscopic analysis of particle abundance

To determine the particle abundance within 2 h of sampling 1 mL of sterilized water with paraformaldehyde at a final concentration of 1% was added to one of the filter folders to fix the aerosols (Maki et al. 2014). After a 1-h incubation period, the filter was stained with 4, 6-diamidino-2-phenylindole (DAPI) at a final concentration of 0.5 μg/mL for 15 min (Porter and Feig 1980). Next, the filter was placed on a slide on a drop of low-fluorescence immersion oil. A second drop of oil was added, and a coverslip was placed on top. The prepared slides were then observed using an epifluorescence microscope (Olympus, Tokyo, Japan) equipped with an ultraviolet excitation system. A filter transect was scanned, and the mineral particles (white particles), yellow particles and bacterial cells on the filter transect were counted. The detection limit of aerosols was below 5×10^3 particles/m^3 of air.

2.2.4 Cloning analysis targeting 16S rDNA sequences

After the sampling aerosol were washed off the filter by shaking with 5 mL of Tris-ethylenediaminetetraacetic acid buffer. After washing, the aerosol were collected by centrifugation at 20,000 ×g for 5 min. Genomic DNA (gDNA) was extracted from the bacterial cell using SDS, proteinase K, and lysozyme as described previously (Maki et al. 2008). The gDNA was purified by phenol–chloroform extraction, chloroform extraction, and ethanol precipitation. Fragments of 16S rDNA (1,450 bp) were amplified from the extracted gDNA by PCR using the following oligonucleotide primers: 27F, 5–AGA GTT TGA TCM TGG CTC AG–3′; and 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 95 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 2 min, for a total of 30 cycles. The PCR amplicons were purified by phenol-chloroform extraction and chloroform extraction, followed by ethanol precipitation. The PCR amplicons of 16S rDNA fragments were cloned into *Escherichia coli* using a commercially prepared vector PCRII-TOPO with a TA Cloning Kit (Invitrogen, CA, USA) according to the manufacturer's protocol. More than 50 clones were obtained from each sample. The nucleotide sequences were determined using a Dye DeoxyTM Terminator Cycle Sequencing Kit (ABI, CA, USA) and a DNA auto sequencing system (ABI, Model 373A) according to the manufacturer's recommended protocol. The M13 reverse primers were used as the sequencing primer. The defined sequences were compared with DDBJ (DNA Data Bank of Japan) database using Basic Local Alignment (BLAST) to analyze bacterial

species compositions. All sequences without chimeras were assigned as operational taxonomic units (OTUs) based on bacterial species with more than 97% similarities. The coverage of the 16S rDNA library was calculated using the formula [1 – (n/N)], where n is the number of clones. The full sequences of the dominant clones were determined using the M13 reverse primer. The Phylogenetic tree was constructed according to the neighbor-joining algorithm using TreeViewPPC (Saitou and Nei 1987).

2.2.5 Accession numbers

DDBJ accession numbers for the 16S rDNA sequences determined in this study are from AB900929 to AB900963 (Table 2.1).

Table 2.1. Phylogenetic affiliation of 16S rDNA gene sequences obtained from clone libraries

Category	Clone number[a]	Numbers of clones[b]	Sampling period[c]	Length (bp)	GenBank accession number	Closest relative	Similarity (%)[d]
Acidobacteria	KZtp1-8	1	1	597	AB900929	Bacterium Ellin6099 (AY234751)	90.3
Actinobacteria	KZtp12-17	4	1,2,12	602	AB900930	Bacterium SCGC AAA071-N11 (JF488663)	94.2
	KZtp3-22	1	3	721	AB900931	*Actinoplanes pyriformis* (AJ277582)	85.6
Cyanobacteria	KZtp1-1	80	1,2,3,4,4,5,6, 7,8,9,10,11	1497	AB900932	*Synechococcus* sp. CC9902 (CP000097)	96.2
	KZtp3-16	13	2,3,6,11	1360	AB900933	*Synechococcus* sp. CC9605 (CP000110)	99.8
	KZtp1-19	5	1,2,7	454	AB900934	BacteriumWHC3-9 (JQ269283)	91.2
	KZtp10-6	4	1,7,10	479	AB900935	*Synechococcus* sp. CC9311 (CP000435)	90.9
	KZtp4-12	1	4	710	AB900936	*Halospirulina* sp. EF17 (2012) (JX644589)	95.5
Firmicutes	KZtp5-8	96	2,4,5,7,10,11, 12	1580	AB900937	*Bacillus megaterium* (DQ789400)	100.0
	KZtp6-42	60	6,8	1459	AB900938	*Bacillus* sp. 4115 (JX566594)	99.9
	KZtp9-16	16	4,6,8,9	1432	AB900939	*Bacillus subtilis* (EF523474)	100.0
	KZtp2-5	7	1,2	1492	AB900940	[Brevibacterium] halotolerans (JX644589)	99.8
	KZtp8-30	4	8,10	1460	AB900941	Bacillus subtilis (EF523474)	99.7
	KZtp6-1	3	6,8	1515	AB900942	*Staphylococcus hominis* (FJ768458)	99.9
	KZtp5-10	2	5,7	613	AB900943	*Bacillus megaterium* (KF419129)	100.0
	KZtp7-43	2	7	600	AB900944	*Bacillus* sp. 6014 (JX566659)	99.3
	KZtp6-38	2	6	596	AB900945	*Bacillus* sp. H69 (KC017473)	99.8
	KZtp8-32	1	8	1462	AB900046	*Bacillus subtilis* (JQ435698)	98.4
	KZtp5-26	1	5	621	AB900947	*Bacillus* sp. M81(KC466182)	99.8
Eukaryota	KZtp4-9	11	1,4,9	710	AB900948	*Quercus nigra chloroplast* (HQ664601)	99.9
	KZtp2-13	11	2,3,4,5	694	AB900949	*Quercus nigra chloroplast* (HQ664601)	99.7
	KZtp7-13	6	7,8	567	AB900950	*Pinus merkusii chloroplast* (FJ899579)	99.8
	KZtp12-26	6	2,6,7,12	599	AB900951	*Pinus pinaster chloroplast* (FJ899583)	100.0
	KZtp3-19	1	3	717	AB900952	*Micromonas* sp. RCC299 chloroplast (FJ858267)	99.3
Proteobacteria	KZtp5-4	21	5,7,11	1547	AB900953	*Sphingomonas paucimobilis* (KC017473)	100.0
	KZtp9-1	15	9	1424	AB900954	Bacterium WHC5-1 (JQ269290)	99.2
	KZtp3-3	12	1,2,3,4,8,9,10	1549	AB900955	Bacterium SH1-7 (JQ269250)	91.6
	KZtp7-24	2	7,11	1498	AB900956	*Sphingomonas paucimobilis* (KC017473)	97.4
	KZtp4-2	2	4	740	AB900957	*Escherichia coli* DH1 (CP001637)	99.9
	KZtp2-23	2	2,4	625	AB900958	*Alpha proteobacterium* IMCC10406 (FJ53493)	99.4
	KZtp5-16	1	5	622	AB900959	*Herbaspirillium aurantiacum* (HQ830497)	99.5
	KZtp5-1	1	5	691	AB900960	*Cupriavidus metallidurans* (JQ65969)	99.6
	KZtp7-37	5	7	599	AB900961	Bacterium SH1-7 (JQ269250)	90.9
	KZtp6-14	3	6	535	AB900962	*Alteromonas macleodii* (CP004855)	92.5
	KZtp1-17	1	1	650	AB900963	*Acinetobacter calcoaceticus* (JX010982)	99.6

[a] Clones of 16S rDNA library were named as the KZtp series.
[b] The number of the clones in 16S rDNA clone libraries.
[c] Sampling period when the air sample was collected
[d] similarity value between each isolate and the closest relative in databases.

2.3 RESULT AND DISCUSSION

2.3.1 Environmental conditions

Relative humidity varied randomly around 40% during the sampling period, and the temperature from May 2 to May 5, 2011 fluctuated at lower values, below 20 ºC, than on May 1 and from May 6 to May 7 (Table 2.2). Some precipitation occurred on May 1, suggesting that a cyclonic flow originating from the western North Pacific or from the East China Sea contributed to rainfall in Kanazawa city on May 1. From the evening of May 1 to the morning on May 7, the days were sunny and calm.

Analyses of air-mass backward trajectories revealed four variations from May 1 to May 7, 2011 (Fig. 2.1). The air mass on May 1, 2011 originated from the North Pacific Ocean and passed the southern or western parts of Japan to Kanazawa city (Fig. 2.1a). In contrast, the air mass between May 2 and May 3 came from the desert area of the Asian continent and passed over the industrial area in China and across the Sea of Japan (Fig. 2.1b). From May 4 to May 5, the air mass was carried from the northern of the sea of Japan or Korea Peninsula to Kanazawa city for three days with low level transport over Korea and Japan (Fig. 2.1c). The air mass from May 6 to May 7 had a similar transport pattern to that of May 1 and came from the southern or western parts of Japan (Fig.2.1d). Changes in aerosol transportation are primarily controlled by the prevailing air flowing from China.

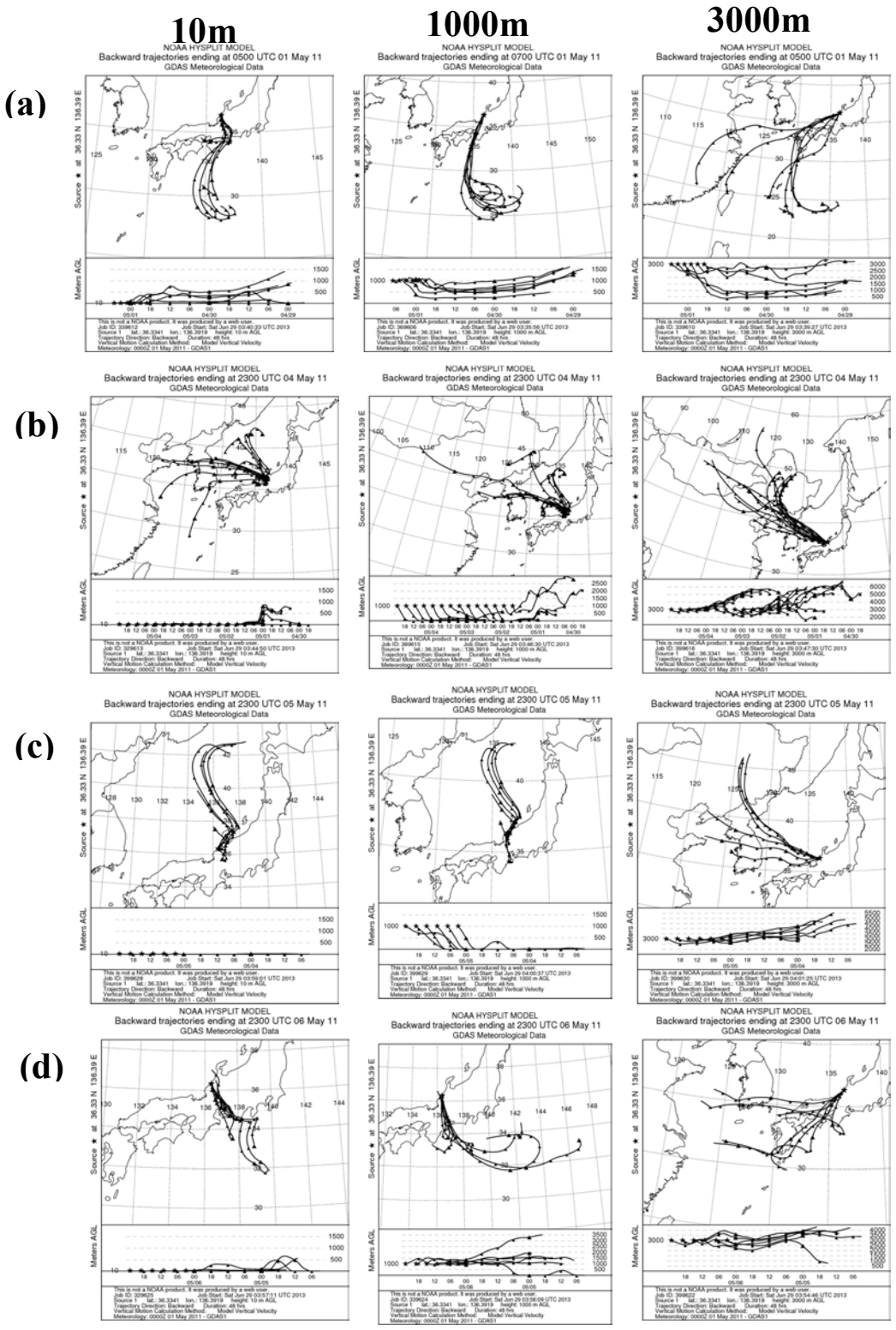


Fig. 2.1 Three-day backward trajectories of aerosols that arrived in Kanazawa city at altitudes of 10, 1,000, and 3,000 m from 5:00 JST to 18:00 JST on May 1, 2011(a), from 18:00 JST on May 1 to 24:00 JST on May 4 (b), from 24:00 JST of May 4 to 24:00 JST on May 5 (c) and from 0:00 JST to 24:00 JST on May 6 (d)

According to lidar measurement at Toyama city, Japan, the depolarization ratio at the ground surface was significantly increased from the evening of May 1 to the evening on May 4, in addition trajectory analysis indicated that the air mass during the period from May 1 to May 4 originated from west. This observation indicated that a long term Kosa event occurred during this time around the Hokuriku area (Fig. 2.3). The Kosa event period is occurred from the evening of May 1 to the evening on May 4.

Kosa carries such as bacteria, fungi, viruses, and mineral particles. Bioaerosols play an important role in microbial dispersal and have a significant impact on ecosystems, human health, agricultural productivity and climate change in downwind areas (Jaenicke 2005; Brown and Hovmøller 2002). Outbreaks of Kosa over East Asian region are very frequent in the spring and last for a few days each time. In this study, the lidar measurements at Toyama city revealed that dust particles were transported to Hokuriku area (Kanazawa city) and that a Kosa event occurred between May 1 and May 4, 2011.

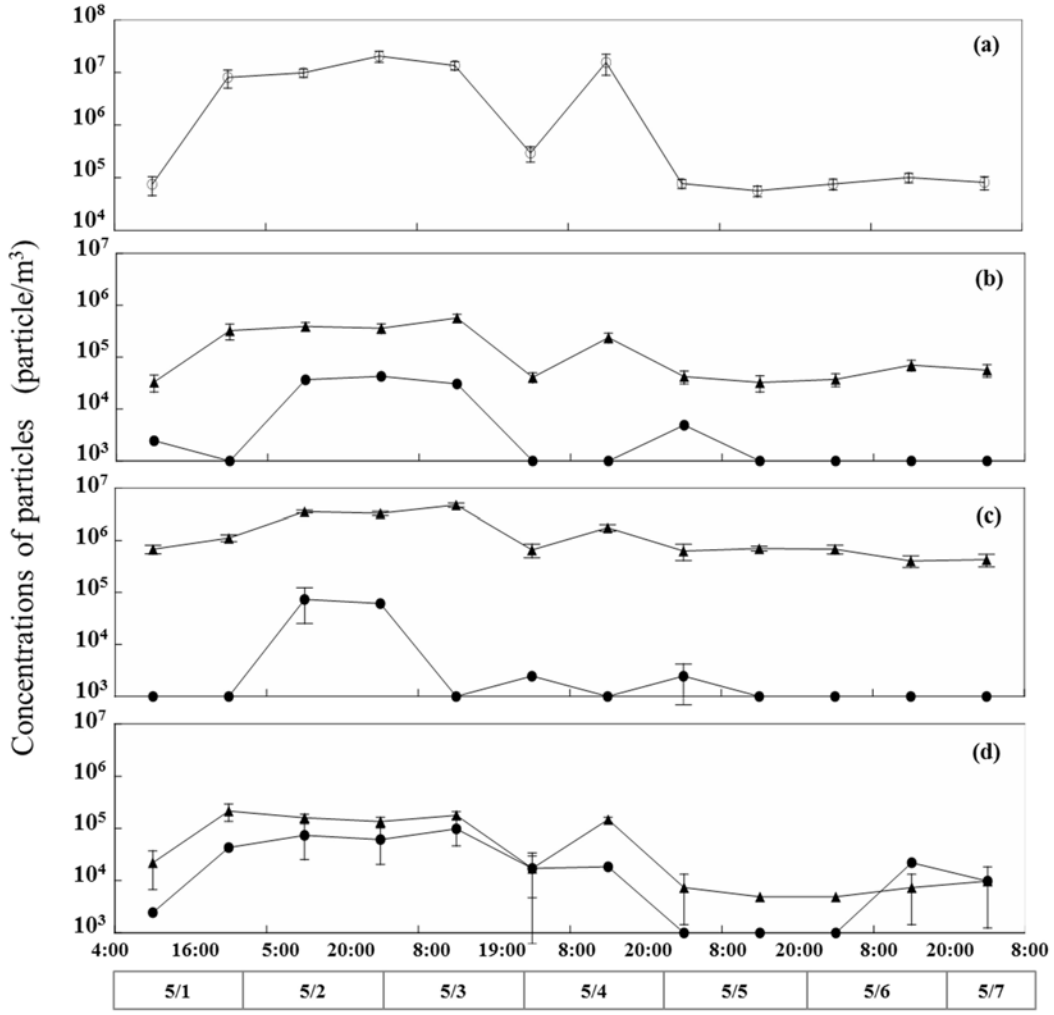


Fig. 2.2 Temporal variations of concentrations of all bacterial particles (open circles) (a), and white particles (closed circles) and yellow fluorescence particles (closed triangles) of 0.2-2.5 μm (b), 2.5-5.0 μm (c), and >5.0 μm in diameter (d) in bioaerosol samples collected at 10 m altitude in Kanazawa city (1 – 7 May, 2011)

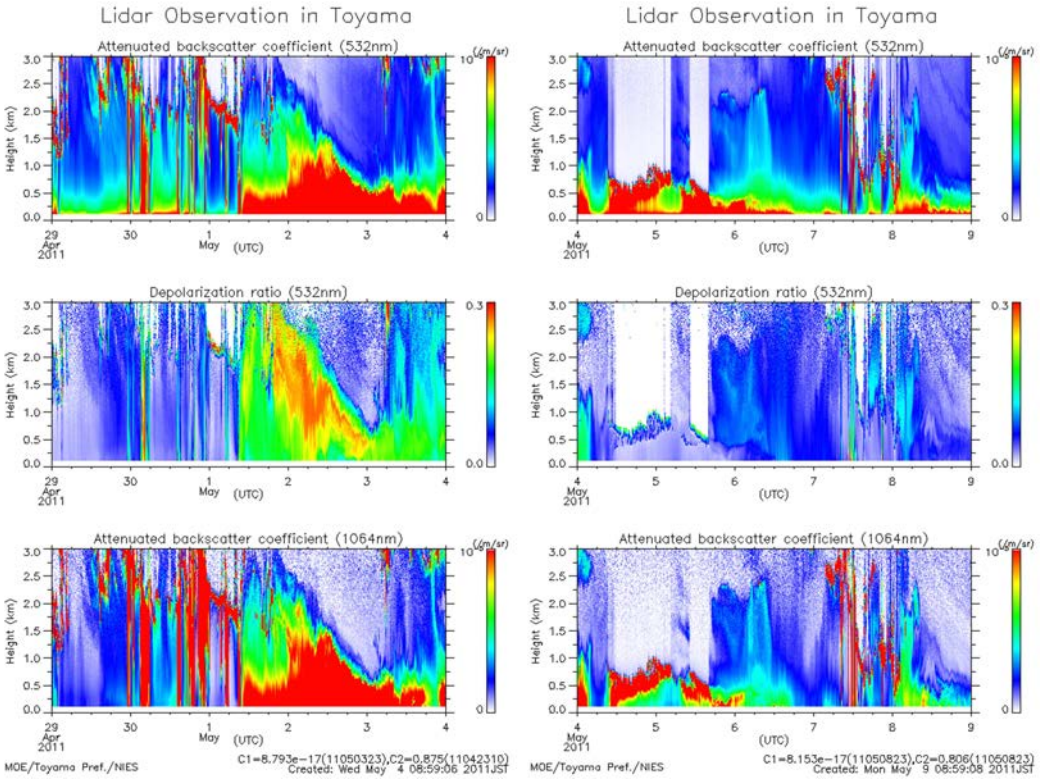


Fig. 2.3 Lidar observation of attenuated backscatter coefficient (532nm), depolarization ratio and attenuated backscatter coefficient (1064nm) in Toyama city from 0:00 in 29 Apr. 2011 to 0:00 in 4 May. 2011 (a) and from 0:00 in 29 Apr. 2011 to 0:00 in 9 May. 2011 (b)

2.3.2 Microscopic observation of aerosols

When DAPI staining of aerosol particles collected at 10 m in Kanazawa city was performed on the sampling filter, the aerosol comprised both separate and aggregated particle. White blue self-fluorescent particles, which were mineral particles, exhibited relatively large sizes, with diameters ranging from 0.2 μm to 100 μm. Yellow fluorescent particles, potentially organic matter, were observed to range from 0.2 μm to 10 μm in diameter. DAPI stained bacteria were observed as coccoid and bacilli like particle with a diameter of < 1.0 μm and bright blue fluorescence. These tree types of particles formed aggregated ranging from 2.0

μm to 100 μm in diameters. This indicated the bacterial particles attached to large particles were transported through the atmosphere.

The total density of bacterial cell in air samples increased from 7.5×10^4 particles m^{-3} to 2.0×10^7 particles m^{-3} during the Kosa event and decreased to 7.7×10^4 particles m^{-3} on non Kosa event day (Fig 2.2a). White blue fluorescent particles with diameters ranging from 0.2 μm to 5.0 μm significantly increased to 4.5×10^6 particles m^{-3} during the Kosa event and decreased to 10^5 particles m^{-3} after the kosa event finished (Fig. 2.2b, c). White blue particles of > 5.0 μm diameters also increased to 7.4×10^4 particles m^{-3} during the Kosa event but were less than 10^3 particles m^{-3} on non Kosa event day (Fig. 2.2d). Yellow fluorescent particles from 0.2 μm to 5.0 μm in diameter fluctuated from concentrations of more than 2.4×10^5 particles m^{-3} to 10^4 particles m^{-3} during non Kosa event days (Fig 2.2b, c). Yellow fluorescent particles of >5.0 μm diameter peaked at approximately 4.3×10^4 particles m^{-3} during the Kosa event and were undetectable on non Kosa event days (Fig 2.2d). The concentration of aggregated particles ranging from 0.2 μm to 5.0 μm in diameter increased to more than 1.4×10^5 particles m^3 during the Kosa event but maintained values in the order of 10^3 particles m^{-3} on non Kosa event days. Larger aggregated particles of >5.0 μm diameters significantly increased to 9.8×10^4 particles m^{-3} during the Kosa event, decreasing to below limited detection on non Kosa event days and increasing to 2.2×10^4 particles m^{-3} at the non Kosa event days May 6 and May 7.

Epifluorescence microscopy demonstrated that the several types of particles such as mineral particles (white particles), organic particles (yellow particles), and microbial particles were present in air samples collected at an

altitude of 10 m. DAPI stained particles with yellow fluorescence have been reported to resemble organic materials originating from proteins and others microbial cell components (Mostajir et al. 1995) and were observed to be present at the altitude of 3000 m over Suzu city Japan during a Kosa event (Maki et al. 2013).

The concentration of aerosol particles increased 10 to 100- fold during the dust event (Fig. 2.1). Kosa events have been reported to increase the biomass of airborne microorganisms in correspondence with the amount of mineral particle (Hara and Zhang 2012) and significantly change bacterial species structures in the free troposphere (Maki et al. 2013). The majority of phylotypes recovered from 12 samples belonged to the phyla *Cyanobacteria*, *Alphaproteobacteria*, and *Firmicutes*, and the bacterial compositions consisting of the members from the three phyla showed significant dynamics from the initiation of the Kosa to the days after the event had passed (Table 2.1, Fig. 2.4).

2.3.3 Dynamics of 16S rDNA clone libraries

The 16S rDNA fragment in the air samples were amplified by PCR using primers targeting eubacteria 16S rDNA. The PCR amplicons were cloned into *E. coli*, and a total of 402 clones including eubacterial 16S rDNA fragment were obtained from the 12 samples. Sequences of the 16S rDNA clones indicated that the airborne bacterial populations were composed of several bacterial species (Table 2.1). Reasonable coverage ranging from 80% to 93% was obtained, indicating that the majority of the airborne bacterial were represented in the libraries. Most of the majority of phylotypes recovered from the air samples

belonged to the phyla *Cyanobacteria*, *Proteobacteria*, and *Firmicutes*, which are typically well represented in 16S rDNA clone libraries generated from terrestrial and marine environment. Only a few bacterial sequences were affiliated with *Actinobacteria* or *Acidobacteria*. The bacterial composition of the clone libraries showed significant dynamics during the sampling period (Fig. 2.4). Only 8 of the total 403 sequenced clones could not be affiliated with any known bacterial group.

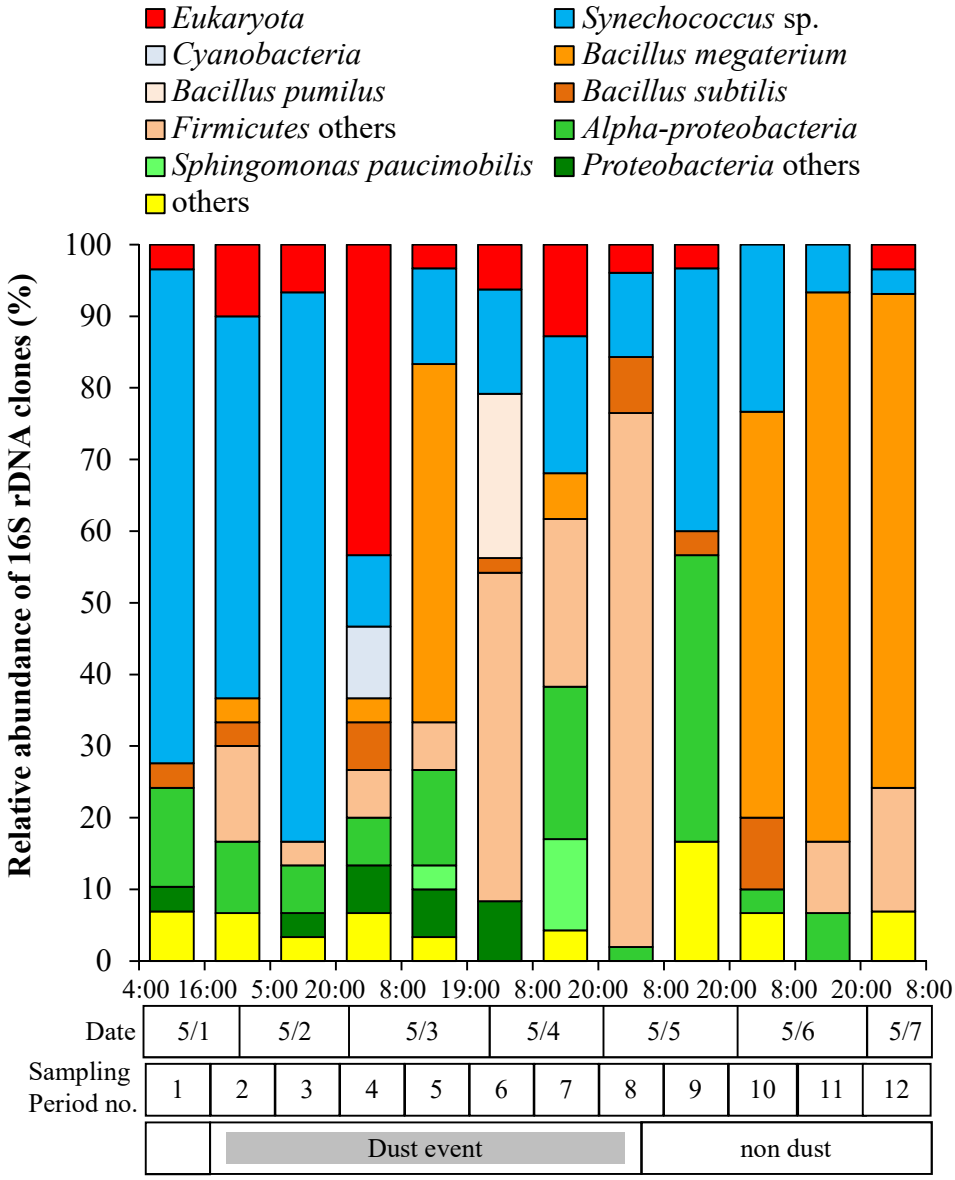


Fig. 2.4 Change in compositions of the partial sequences of 16S rDNA clones (Ca. 400 bp) obtained from air samples collected at 10 m altitude in Kanazawa city (1 – 7 May, 2011)

Of the sequences clones derived from all 12 samples, 27% were from *Cyanobacteria* (Table 2.1) and formed two distinct groups in the marine type cluster of the genus *Synechococcus* in the phylogenetic tree (Fig. 2.5). One group was composed of clones detected from Kosa event samples (Samples 3 and 5; Fig. 2.4). Sequences from this group clustered with the coastal and ocean *Synechococcus* spp. found in warm areas and were closely related to strains previously isolated from the Sea of Japan. In contrast, the sequences of the others group were detected in every sample and were mainly related to a coastal *Synechococcus* species found in cold environments.

More than 46.5% of the clones were from *Firmicutes* (Fig. 2.4), almost all of which were related to members from the genera *Bacillus* and *Staphylococcus* (>99.7% similarity). The complete 16S rDNA sequences of the *Bacillus* species has high similarities (>99.7%) with *Bacillus subtilis*, *Bacillus pumilus* and *Bacillus megaterium* (Fig. 2.6). Some of the *B. subtilis* and *B. pumilus* clones appeared specifically at night during the Kosa event (Samples 4 and 6) and were closely related with isolates detected at high altitudes above Suzu city during a Kosa event (Maki et al. 2008). Other clones belonging to the genus *Bacillus* were closely related to *B. megaterium* and were predominant in Samples 10, 11, and 12, which were obtained after the Kosa event has finished. Several minor clones were related to *Staphylococcus hominis* and increased in Sample 6, which was obtained during Kosa event.

Clones from *Proteobacteria* comprised 13.3% or the total clones (Table 2.1) and were mainly clustered in the genus *Sphingomonas* and the *alphaproteobacteria* SAR clade (Fig. 2.7). The clones affiliated with

Sphingomonas spp. were detected in almost all samples, and their numbers significantly increased at the end phase of Kosa event (Sample 9; Fig. 2.4). Some clones showed 99.8% similarity with some species of *Sphingomonas* found in the northern sea, whereas others clones were closely related to *Sphingomonas paucimobilis*. Clones from the *Alphaproteobacteria* SAR clade, which contains species unique to ocean environment at the Sea of Japan and Pacific Ocean had low similarities (<97.1%) with other members of the SAR clade, suggesting that these clones represent novel bacterial species in the SAR clade. These sequences appeared randomly throughout the Kosa event as <10%of the total clones in each sample (Samples 5 and 6) and significantly increased to 50% of the total after the Kosa event finished (Sample 8).

2.3.4 Dominated bacterial species in the air sample

Cyanobacteria

Phylogenetic analysis showed that all the clones from *Cyanobacteria* formed two clusters in the marine genus *Synechococcus* spp. found in the Sea of Japan and East China Sea (Choi and Noh 2009) and coastal areas (Fuller et al. 2003; Fig 2.5). Kosa particles reaching Japan are mixed with seawater compounds when passing over the Sea of Japan (Zhang et al. 2006). *Cyanobacteria* including *Synechococcus* spp., are known to resistant to UV irradiation and oxygen stress because they have to eliminated excess peroxide generated from photosynthesis (Latifi et al. 2009; Perelman et al. 2003). In a previous study, marine microorganisms such as *Cyanobacteria* were shown to be transported by a Kosa event and comprised 20% of the clones libraries obtained from air samples

collected from Europe regions (Polymenakou et al. 2008). Clones affiliated with *Synechococcus* spp. dominated Sample 1 and 2, which had been collected during the first phase of Kosa event, and then decreased to no less than 30% during the sampling periods (Fig 2.4). It is likely that the front air mass of the Kosa event coming from the continental area would have blown the cells of marine *Synechococcus* spp., as well as sea water, up into the air; the cells and the seawater would subsequently have fallen down upon the downwind area.

Firmicutes

Sequences related to the *Firmicutes* members *B. subtilis* and *B. pumilus* comprised most of the 16S rDNA clones libraries from samples 6 and 8 (Fig 2.4 and 2.6), which had been taken at night during the Kosa event. This sample contained higher concentrations of aerosol particles than the others samples, in accordance with the occurrence of a Kosa event (Fig. 2.2). Some clones showed more than 99.7% similarity with *B. subtilis* and *B. pumilus,* which were predominant among aerosol collected at high altitudes above the Taklamakan desert (Maki et al. 2008), from an downwind area of Kosa even (Suzu city; Maki et al. 2010), and from the mineral particles collected from snow fall at Mountain Tateyama (Maki et al. 2011). Species related to *B. subtilis* were isolated from sand from Gobi desert (Hua et al 2007) and are reported to dominated in the surface air of Soul city during Kosa event (Jeon et al. 2011). It has been shown that *Bacillus* spp. carried by Kosa event were predominant in air sample taken during a free-tropospheric sampling carried out on a North America mountain (Smith et al. 2012). It has been shown that *Bacillus* spp. form resistant endospores to enhance

their survival in the atmospheres (Nicholson et al. 2000). Presumably, at night during the Kosa events, microbial particles such as *B. subtilis* and *B. pumilus* fell from the free troposphere to near ground surfaces levels in Kanazawa city.

Unlike to *B. subtilis* and *B. pumilus,* the *Firmicutes* species *B. megaterium* was found in the daytime during the Kosa event and dominated after the Kosa event finished. *B. megaterium* has rarely been detected at high altitudes above the Taklmakana Desert and Kosa arrival areas (Suzu city and Mt. Tateyama; Hua at al. 2007; Kakikawa et al 2009). Possibly, in the daytime, the upward flow of air caused by sunlight may contaminate the surface atmosphere with the local bacteria population from ground surfaces. The trajectories indicated that after the Kosa event finished, the air mass mainly remained around Japan for a few days. Thus, it is possible that the *B. megaterium* detected in the sample originated from the local ground surface and was transported to the airborne bacterial communities by upper flow.

Alphaproteobacteria

The proportion of the clone libraries representing the *Alphaproteobacteria* members of the SAR clade and the genus *Sphingomonas* fluctuated from 135 to 25% and increased at the end phase of Kosa event and after the Kosa event finished (Samples 5, 6, and 9; Fig. 2.4 and 2.7). Members of SAR clade (Giovannoni and Stingl, 2005) and *Sphingomos* spp. (Eguchi et al. 1996) are known to be ubiquitous marine oligotrophic ultra microbacteria, which are thought to demonstrate improved survival at low substrate concentration. The SAR clade is composed of ubiquitous and unculturable marine bacteria detected in

the Sea of Japan (Song et al. 2009) and in ocean area such as the Pacific Ocean (Giovannoni and Stingl 2005). Member of *Pelagibacter*, including the SAR clade, occupy approximately 25-50% of marine bacteria in ocean areas, indicating their ability to survive in extreme environment (Morris et al. 2002). *Sphingomonas* spp. have also been identified as being an important part of marine bacterial plankton and are often found in marine bacterial communities from North Asian areas (Dieser et al. 2010) and the polar regions (Gloeckner et al. 2000). *Sphingomonas* spp. are often found to comprise the dominant bacterial population in the free troposphere over Noto peninsula, Japan (Maki et al. 2013) and in cloud water (Amato et al. 2007). *Sphingomonas* spp. have been reported to be particularly resistant to elevated concentrations of oxidants such as hydrogen peroxide, one of the major sources of free radicals in cloud water, and to have the capacity to rapidly adapt to changing nutritive conditions (Eguchi et al 1996; Ostrowski et al. 2001). Furthermore, some strains of *Sphingomonas* spp.. Have been repeatedly isolated from extreme and cold environments such as Arctic and Antarctic soils (Baraniecki et al. 2002) and Greenland ice core (Miteva et al. 2004). Bacterial populations that are resistant to various environment stressors in extreme environments, such as oceans or cold regions, would be able to extent their habitats efficiently via atmospheric transport.

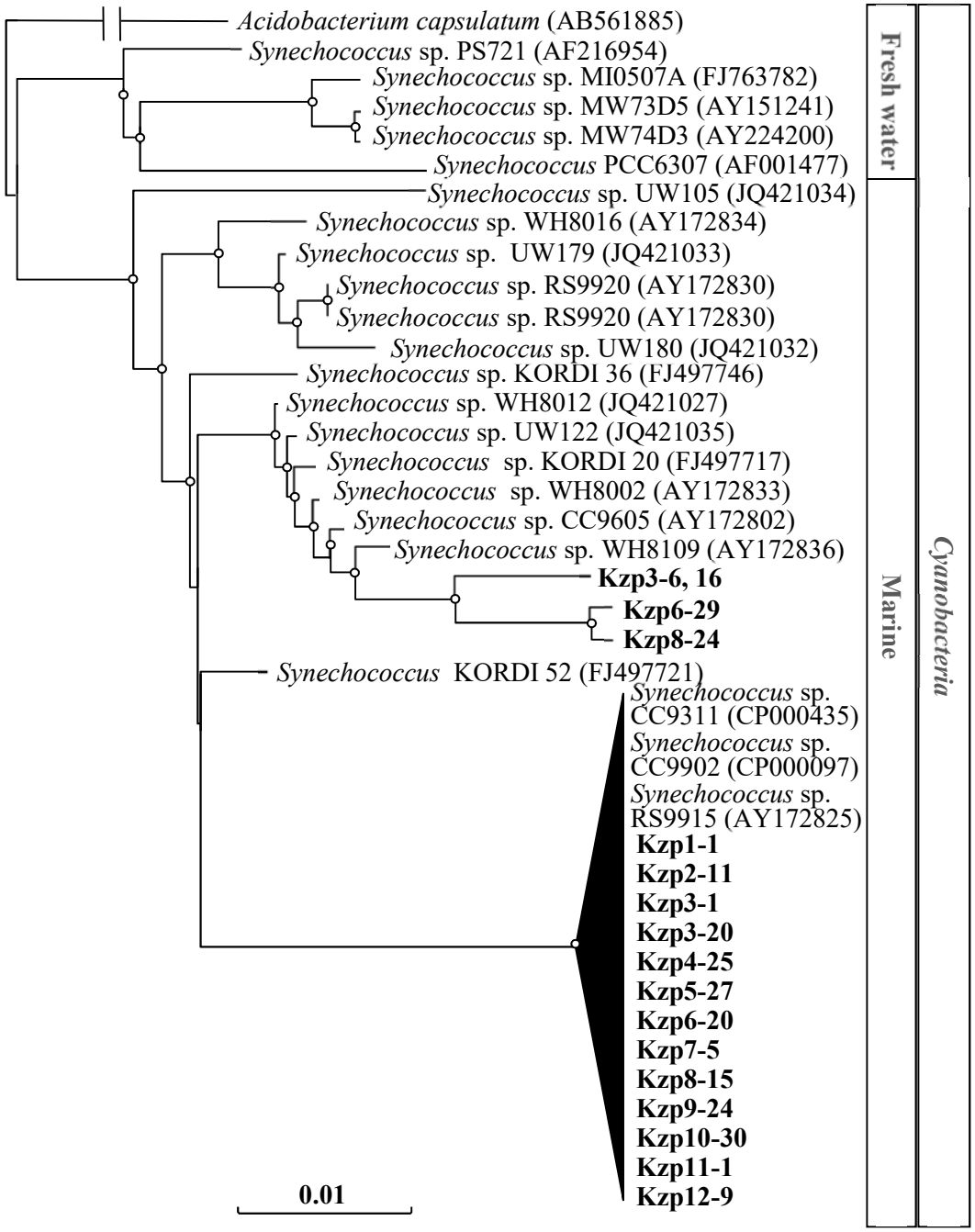


Fig. 2.5 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained from the clone libraries (Kzp series) from the bioaerosol samples collected in Kanazawa city and the known members of *Cyanobacteria*. The phylogenetic tree was calculated from a dissimilarity matrix of an approximately 1400 bp alignments (*E. coli* numbering 92 to 1475) 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 resampling)

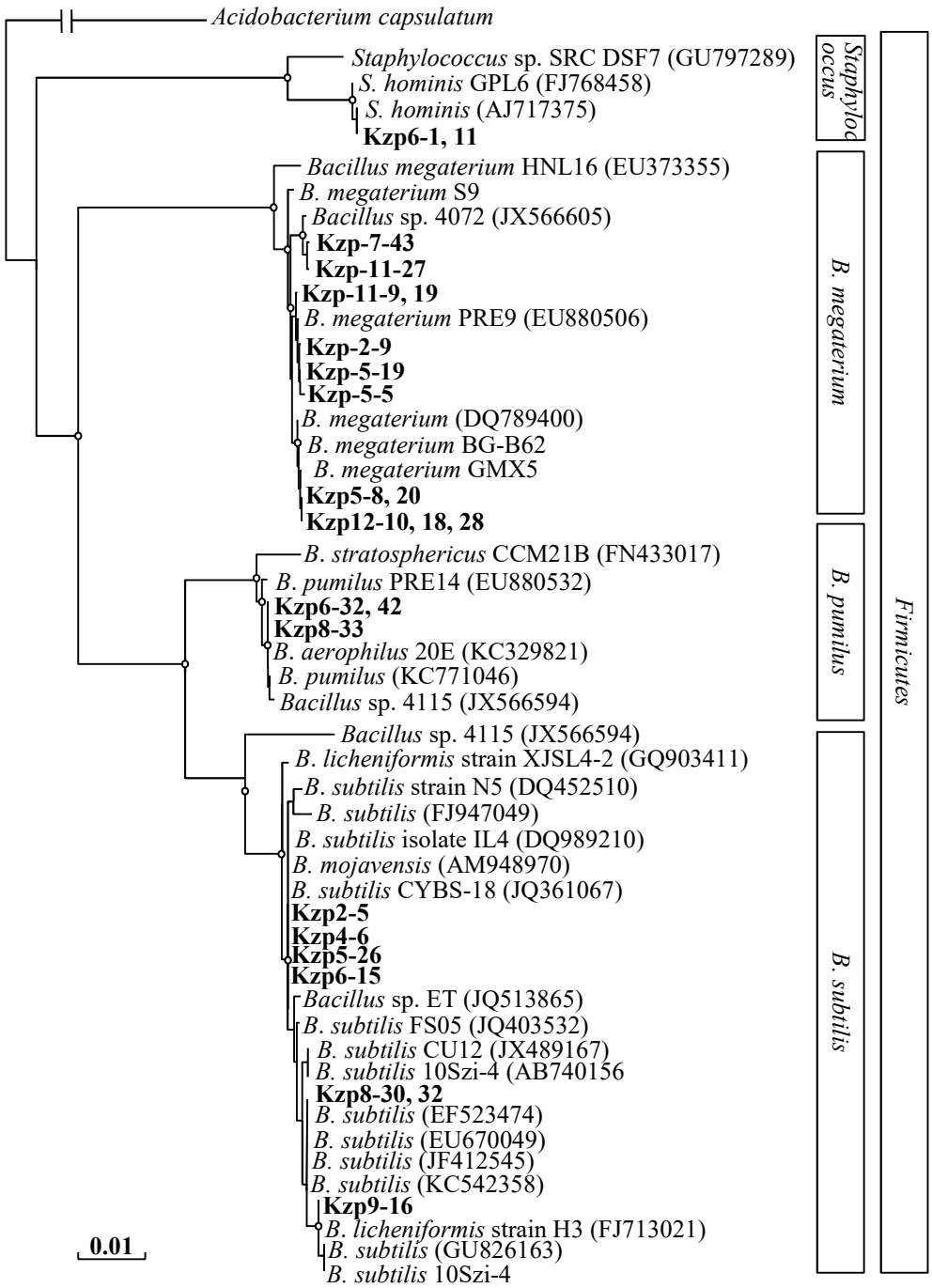


Fig. 2.6 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained from the clone libraries (Kzp series) from the air samples collected in Kanazawa city and the known members of *Firmicutes*. The phylogenetic tree was calculated from a dissimilarity matrix of an approximately 1400 bp alignments (*E. coli* numbering 71 to 1432) 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 resampling)

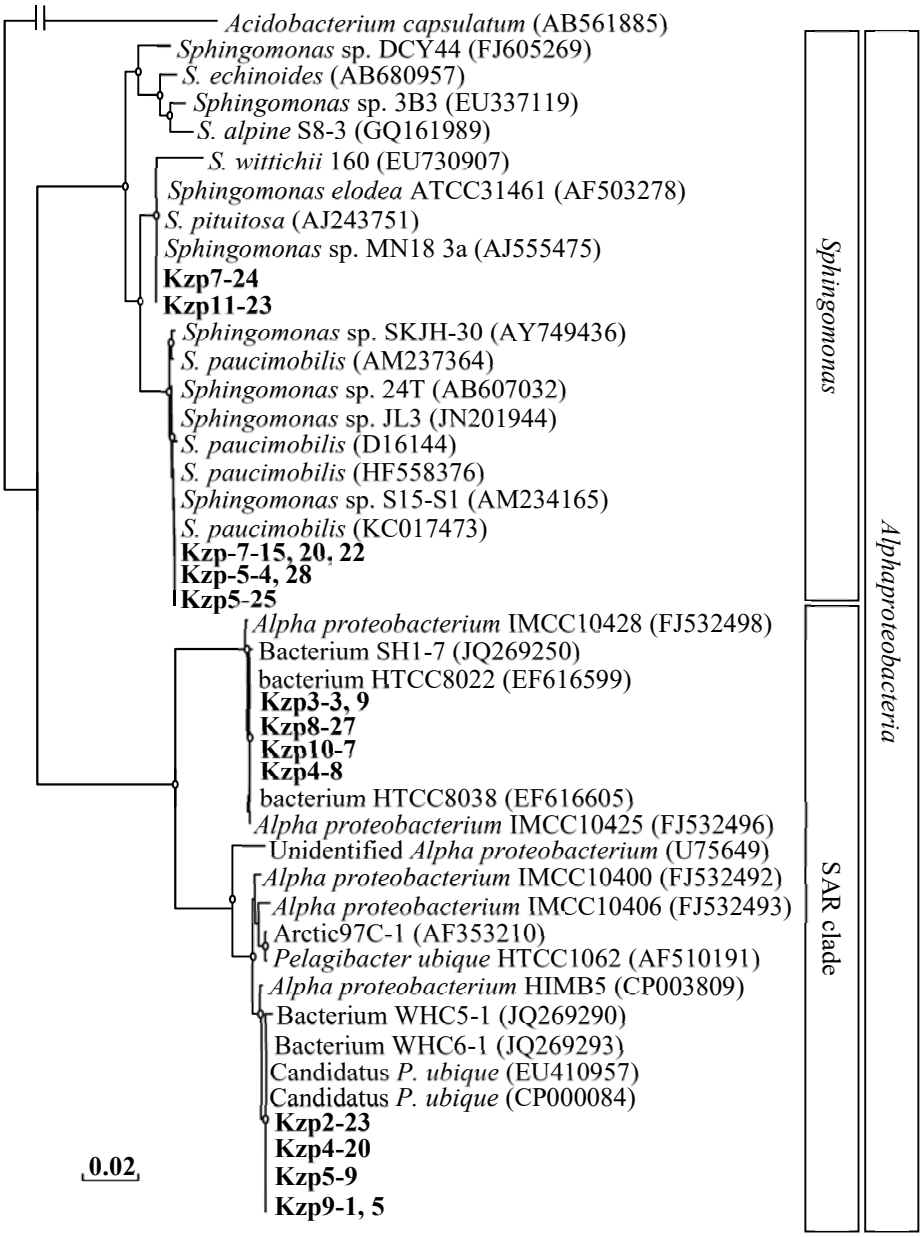


Fig. 2.7 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained fromthe clone libraries (Kzp series) from the air samples collected in Kanazawa city and the known members of *Alphaproteobacteria*. The phylogenetic tree was calculated from a dissimilarity matrix of an approximately 1400 bp alignments (*E. coli* numbering 86 to 1437) 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 resampling)

Table 2.2 Meteological conditions from April 30 to May 7, 2011, in Kanazawa city and Wajima city

		Kanazawa City (36.35°N, 136.38°E)							Wajima City (37.23°N, 136.57°E)						
Day	Time	Temperature (°C)	Precipitation (mm)	Humidity (%)	Wind velocity (m/s)	Atmospheric pressure (hPa)	Dew point temperature (°C)	Steam pressure	Temperature (°C)	Precipitation (mm)	Humidity (%)	Wind velocity (m/s)	Atmospheric pressure (hPa)	Dew point temperature (°C)	Steam pressure
April 30	1	10.6	--	57	2.2	1013.6	2.4	7.3	10.6	--	69	4	1016.1	5.2	8.8
	2	10.8	--	56	1.3	1013.3	2.4	7.3	10.9	--	62	5.3	1016.1	3.9	8.1
	3	11	--	54	2.4	1012.8		7.1	11.4	--	56	4.2	1015.8	3	7.5
	4	10.5	--	63	1	1012.5	3.8	8	10.8	--	62	4	1015.5	3.8	8
	5	10.8	--	63	2.1	1011.8	4.2	8.2	10.3	--	67	3.6	1014.8	4.5	8.4
	6	11.4	--	61	1.6	1011.7	4.2	8.2	11.6	--	60	4.5	1014.3	4.1	8.2
	7	12	--	60	3.3	1011.3	4.5	8.4	12.4	0	58	4.7	1014.2	4.4	8.4
	8	15.9	0	43	3	1010.4	3.3	7.7	11.4	0	77	4.2	1013.2	7.5	10.4
	9	13.3	0	70	1.8	1010.5	8	10.7	15.8	0	52	3.3	1012.8	6	9.3
	10	13.9	0	70	3.2	1009.3	8.2	10.9	12.8	1	74	4.1	1012.4	8.3	10.9
	11	19	--	44	0.9	1007.7	6.5	9.7	12.6	0	76	7.2	1011.5	8.5	11.1
	12	22.3	0	29	8.6	1007.3	3.4	7.8	11.6	0.5	86	2.3	1013.1	9.4	11.7
	13	18	1.5	53	5.9	1007.1	8.4	11	12.2	1	88	2.4	1013.4	10.3	12.5
	14	15.8	0	67	5.9	1010.3	9.7	12	12.2	2	88	4	1011	10.3	12.5
	15	14.7	1.5	79	6.1	1008.5	11.3	13.4	14.2	0	74	12	1007.5	9.7	12
	16	18.4	0	62	3.9	1005	11.2	13.3	15.6	--	72	8	1007.4	10.6	12.8
	17	22.6	--	41	8.7	1004.1	8.7	11.2	17.3	--	66	4	1006.8	10.9	13
	18	22.8	--	41	7.1	1003.3	8.9	11.4	19.3	--	55	8.2	1006.9	10.1	12.3
	19	22.4	--	41	5.9	1004.2	8.5	11.1	17.7	--	64	4.9	1007.1	10.8	13
	20	21.8	--	43	7.4	1005.6	8.6	11.2	20.8	--	48	7.9	1006.9	9.4	11.8
	21	22.4	--	40	9.4	1005.8	8.5	11.1	20.9	--	47	8.3	1007.8	9.2	11.6
	22	23.4	--	40	7.6	1005.7	9.1	11.5	20.7	--	47	7.5	1008.2	9	11.5
	23	22.5	0	42	9.2	1006.2	9	11.4	21.2	0	50	7.3	1008	10.4	12.6

	24	20.4	0	61	5.6	1005.7	12.6	14.6	17.9	0.5	67	7.5	1008.5	11.7	13.7
May 1	1	20.3	0	55	6.5	1004.9	10.9	13	17.1	0	74	5.4	1007.7	12.4	14.4
	2	20.8	0	50	7.3	1004.8	10	12.3	18	0	70	5.7	1007.3	12.5	14.4
	3	19	0	64	4.9	1004.5	12	14.1	16.6	0.5	76	7.7	1007.1	12.4	14.4
	4	17.3	0	76	2.9	1003.9	12.8	14.8	16.1	1	82	6.5	1006	13	15
	5	15.9	1	88	1.4	1002.3	13.8	15.7	16	1	84	5.6	1005	13.3	15.3
	6	19.3	0	62	5.3	1000.1	11.9	14	15.6	1.5	85	8.5	1003.6	13.1	15.1
	7	17.7	0	76	3.3	1000	13.3	15.3	15.8	0.5	80	8.5	1002.6	12.4	14.4
	8	21.2	0.5	52	9.5	999.1	11	13.1	15.9	2	80	8.2	1002.3	12.5	14.5
	9	21.3	0	48	16.2	998.2	9.9	12.2	17.4	0	75	9	1000.9	12.9	14.9
	10	23.3	0	44	15.5	997.3	10.3	12.5	19.1	0	66	11.5	1000	12.6	14.6
	11	23.3	0	44	14.1	996.8	10.4	12.6	20	0	63	8	999.3	12.8	14.7
	12	23.3	0	44	13.2	996.7	10.4	12.6	23.4	--	48	9	998.3	11.8	13.8
	13	22.9	0	45	12.4	995.6	10.4	12.6	23.6	--	48	9.2	997.1	12	14
	14	22.6	0	47	11.1	994.7	10.7	12.9	22.5	--	51	11.2	996.3	11.9	13.9
	15	22.6	0	48	11.2	994	11.1	13.2	21.9	--	53	9.7	996.2	11.9	13.9
	16	22.7	0	48	10.9	994.3	11.1	13.2	20.8	--	60	9.3	996.7	12.8	14.7
	17	22.9	0	52	9.1	995.3	12.5	14.5	16.1	1.5	84	8.6	998.7	13.4	15.4
	18	17.2	1.5	70	8.2	998.3	11.5	13.5	15.9	0.5	79	8.2	999.5	12.3	14.3
	19	17.1	0	79	6.9	1000.5	13.4	15.4	14.7	--	78	8.4	1000.6	10.9	13
	20	16.2	0	76	7.9	1002.1	12	14	14.1	--	78	6.9	1002.7	10.3	12.5
	21	15.8	0	74	7.2	1003.1	11.4	13.5	13.6	--	77	6.1	1003.9	9.7	12
	22	15.3	0	76	5.9	1003.5	11.2	13.3	12.8	--	77	5.8	1004.3	8.9	11.4
	23	14.5	0	80	6.3	1004.4	11.1	13.2	12.7	--	67	6.7	1004.8	6.7	9.8
	24	14.4	0	75	6.4	1005	10.1	12.4	12.8	--	67	6.8	1005.9	6.8	9.9
May 2	1	14.9	0	55	6.1	1005.8	6	9.3	13.1	--	65	7.5	1007.1	6.7	9.8
	2	14.1	0	60	5.2	1006.4	6.5	9.7	12.1	--	71	3.6	1008.2	7	10
	3	13.3	0	63	4.8	1007.3	6.4	9.6	11.5	--	73	3.6	1009.2	6.8	9.9
	4	13	0	64	4	1007.4	6.4	9.6	10.8	--	75	2.6	1010.1	6.6	9.7

	5	12.8	0	65	3.2	1007.8	6.4	9.6	9.6	--	83	1.5	1010.6	6.9	9.9
	6	12.2	0	71	1.1	1008.2	7.2	10.2	9.7	--	84	1.9	1011.2	7.1	10.1
	7	13.2	0	66	0.5	1008.3	7	10	11.7	--	77	0.8	1011.2	7.8	10.6
	8	15.4	0	61	1.4	1008.3	8.2	10.8	12.4	--	71	2.9	1011.5	7.3	10.2
	9	15.6	0	58	2.3	1008.4	7.1	10.1	13.3	--	67	2.7	1011.6	7.3	10.2
	10	15.5	0	58	2.8	1008.6	7.3	10.2	14.7	--	61	3	1011.7	7.3	10.2
	11	16.7	0	66	3	1008.5	10	12.3	15.5	--	57	3.9	1011.4	7	10
	12	16.1	0	60	3	1008.3	8.3	11	17.3	--	51	3.9	1010.8	7.1	10.1
	13	16.8	0	57	2.8	1008.6	8.5	11.1	17.7	--	45	3.5	1010.9	5.6	9.1
	14	17	0	60	2	1008.5	9.5	11.9	17.6	--	46	3.5	1011.2	5.9	9.3
	15	16.3	0	56	4.5	1008.5	7.2	10.1	16.8	--	49	4.4	1011.4	6	9.4
	16	15.8	0	65	3	1008.7	9.2	11.6	15.7	--	55	3.6	1011.7	6.7	9.8
	17	15.4	0	57	2.8	1009	7.5	10.4	15	--	58	3.4	1011.9	6.8	9.9
	18	14.8	0	66	2.9	1008.7	8.3	10.9	14.1	--	63	1.7	1011.7	7.2	10.1
	19	14.6	0	65	1.4	1008.8	8.1	10.8	12.7	--	69	1.5	1012.1	7.2	10.1
	20	14	0	72	1.1	1009.2	8.9	11.4	10.7	--	81	1.6	1012.8	7.6	10.4
	21	13.8	0	72	1.7	1009.7	8.9	11.4	9.5	--	85	2.2	1013.3	7.1	10.1
	22	12.8	0	74	2	1010	8.3	10.9	8.9	--	86	2.5	1013.6	6.7	9.8
	23	12.1	0	77	1.6	1009.7	8.2	10.9	8.3	--	87	2.5	1013.2	6.3	9.5
	24	11.5	0	76	1.8	1009.6	7.4	10.3	8.3	--	87	2.8	1013.1	6.3	9.5
May 3	1	11.3	0	79	1.7	1009.3	7.7	10.5	9.2	--	89	3.4	1012.9	7.5	10.4
	2	10.7	0	82	2.2	1009	7.8	10.6	9.3	--	88	2.9	1012.5	7.4	10.3
	3	10.2	0	83	1.8	1008.6	7.5	10.3	8.6	--	89	2.9	1012.2	6.9	9.9
	4	9.8	0	81	1.4	1008.4	6.7	9.8	8.5	--	87	3.4	1012	6.5	9.7
	5	9.8	0	80	3	1008.4	6.5	9.7	8.7	--	86	2.8	1011.9	6.5	9.7
	6	10.5	0	74	0.3	1008.6	6.3	9.5	9.7	--	82	3.6	1012.1	6.8	9.9
	7	11.2	0	73	1.3	1009	6.6	9.7	11.5	--	73	3.6	1012.4	6.8	9.9
	8	13.2	0	65	1.3	1008.9	6.2	9.5	13.4	--	64	4	1012.4	6.7	9.8
	9	14.7	0	54	0.4	1008.6	5.2	8.9	14.1	--	65	2.1	1011.8	7.6	10.5

	10	15.8	0	54	1.4	1008.2	6.9	9.9	15.1	--	60	1.9	1011.8	7.4	10.3
	11	18.2	0	53	2.3	1008.2	8.4	11	14.8	--	60	2.8	1011.3	7.1	10.1
	12	16.7	0	52	3.6	1007.5	7.3	10.2	16.6	--	47	2.3	1010.9	5.3	8.9
	13	16.5	0	53	3	1006.9	6.8	9.9	15.2	--	65	1.4	1010.1	8.7	11.2
	14	15	0	73	4.4	1007.4	10.1	12.4	15.2	--	60	2.2	1009.6	7.5	10.4
	15	15.3	0	64	3.1	1007.6	8.9	11.4	16.6	--	51	1.9	1009.2	6.4	9.6
	16	16.6	0	57	3.9	1007.3	8.4	11	14.9	--	66	4.3	1009.9	8.6	11.2
	17	17.3	0	58	3.8	1006.3	8.9	11.4	14.2	--	73	4.8	1010.5	9.4	11.8
	18	17.6	0	60	1.5	1005.9	9.6	12	13.5	--	74	1.3	1010	9	11.5
	19	15.2	0	75	3.4	1006.5	10.8	13	12.8	--	77	0.9	1009.9	8.9	11.4
	20	14.2	0	77	3.1	1007.2	10.1	12.4	12.3	--	74	3.5	1010.2	7.8	10.6
	21	14.5	0	75	0.9	1007.3	10.1	12.4	11.7	--	75	1.4	1011	7.4	10.3
	22	13.8	0	80	1.5	1007.7	10.4	12.6	10.3	--	80	2.8	1010.8	7	10
	23	13.9	0	66	3	1007.8	7.7	10.5	9.3	--	81	2.8	1011	6.2	9.5
	24	12.6	0	71	3.1	1008.1	7.5	10.4	8.7	--	82	3.3	1010.7	5.8	9.2
May 4	1	12.5	0	65	2	1007.7	6.1	9.4	8.6	--	80	3.7	1010.3	5.4	8.9
	2	12.2	0	61	2.5	1007.6	4.9	8.7	9.2	--	76	3.3	1009.9	5.2	8.8
	3	12.1	0	58	3.8	1007.4	4	8.2	9.1	--	76	2.8	1009.9	5.1	8.8
	4	12.6	0	53	3.3	1007.4	3.3	7.7	8.2	--	81	1.6	1010.2	5.2	8.8
	5	12	0	54	3.1	1007.1	3	7.6	8.2	--	81	0.8	1010.9	5.2	8.8
	6	12.7	0	62	3.5	1008.5	5.5	9	9.2	--	81	0.9	1011.7	6.1	9.4
	7	14	0	72	3.8	1009.8	9.3	11.7	12.5	--	76	0.9	1012.2	8.4	11
	8	14.3	0	65	3.6	1010.5	7.6	10.4	13.9	--	68	2.8	1012.9	8.1	10.8
	9	16	0	69	3	1010.8	10.2	12.5	14	--	65	3.1	1013.8	7.5	10.4
	10	16.2	0	71	4.9	1011.7	11.1	13.2	13.1	--	74	4.2	1014.7	8.6	11.2
	11	16.6	0	65	3.6	1011.5	9.8	12.1	13	--	68	3.1	1014.8	7.2	10.2
	12	16.6	0	52	4	1011.6	7.1	10.1	12.7	--	68	5.2	1014.8	7	10
	13	15.7	0	65	3.6	1011.5	8.9	11.4	11.4	--	74	3.5	1015	6.9	10
	14	14.8	0	70	3.3	1011.6	9.4	11.8	11.4	--	72	3.1	1015.4	6.5	9.7

	15	13.8	0	70	4.7	1011.9	8.4	11	11.2	--	73	2.5	1015.2	6.6	9.7
	16	12.4	0	73	4.8	1012.5	7.7	10.5	10.9	--	75	2.8	1015.9	6.7	9.8
	17	12	0	74	3.8	1012.9	7.5	10.4	10.4	--	75	2.9	1016.4	6.2	9.5
	18	11.8	0	75	4.3	1012.6	7.6	10.4	10.1	--	74	2.9	1016.2	5.7	9.1
	19	11.1	0	77	4.5	1013.4	7.2	10.2	10	--	75	2.4	1016.8	5.8	9.2
	20	11	0	76	3.3	1014.4	6.9	9.9	10.1	--	74	2.3	1017.8	5.7	9.1
	21	10.8	0	76	3.3	1014.8	6.8	9.8	10.1	--	73	2	1018.2	5.5	9
	22	10.8	0	76	3.5	1014.7	6.8	9.8	10.2	--	73	1.4	1018.3	5.6	9.1
	23	10.7	0	78	2.4	1014.5	7	10	9.8	--	79	1.7	1018.2	6.3	9.6
	24	10.8	0	79	1.5	1014.5	7.3	10.2	9.7	--	78	0.8	1018.3	6.1	9.4
May 5	1	10.9	0	80	2.4	1014.2	7.5	10.4	9.6	--	81	1.2	1018.3	6.5	9.7
	2	10.7	0	82	1.6	1014.3	7.8	10.6	10	--	72	2.6	1017.9	5.2	8.8
	3	10.7	0	80	3.8	1013.9	7.4	10.3	9.9	--	70	3	1018	4.7	8.5
	4	10.5	0	80	4.2	1013.8	7.2	10.2	9.8	--	70	3.8	1017.9	4.6	8.5
	5	10.2	0	82	4.3	1014.2	7.3	10.2	9.9	--	73	3.9	1018.4	5.3	8.9
	6	10.2	0	80	4.7	1014.5	6.9	10	10	--	71	3.1	1018.7	5	8.7
	7	11	0	74	4.4	1015.2	6.6	9.7	10.4	--	70	2.5	1019.5	5.2	8.8
	8	12	0	72	4.3	1015.4	7	10	10.9	--	68	2.8	1019.5	5.2	8.9
	9	13.5	0	63	5.9	1015.2	6.6	9.7	11.9	--	69	3.2	1019.6	6.4	9.6
	10	14.3	0	57	5.3	1014.8	6	9.4	12.1	--	67	4	1019.3	6.2	9.5
	11	15.1	0	55	5.9	1014.7	6.5	9.7	13.2	--	67	5.1	1019.3	7.2	10.2
	12	16.5	0	49	5.2	1014.6	6	9.4	13.7	--	63	5.1	1019.2	6.8	9.9
	13	16.1	0	55	6.7	1014.8	7.2	10.2	13.5	--	63	5.2	1019.3	6.6	9.7
	14	16.2	0	59	6	1014.6	8.1	10.8	13.8	--	65	5.8	1019.1	7.4	10.3
	15	15.7	0	61	6.8	1014	8.3	11	13.4	--	66	6.1	1019	7.2	10.1
	16	15.8	0	63	6.5	1013.9	8.8	11.3	12.7	--	70	5.5	1018.6	7.4	10.3
	17	16.3	0	52	5.6	1014	6.5	9.7	11.8	--	70	6.2	1018.8	6.5	9.7
	18	14.1	0	63	6.4	1014.2	7.4	10.3	11.1	--	74	6	1018.9	6.7	9.8
	19	12.4	0	73	6.7	1014.6	7.7	10.5	10.4	--	76	5.5	1019	6.4	9.6

	20	11.6	0	77	6.7	1015	7.7	10.5	10	--	75	2.8	1019.4	5.8	9.2
	21	11.5	0	79	5.9	1015.6	8	10.7	8	--	86	1.6	1020.2	5.8	9.2
	22	11.3	0	81	5	1015.4	8.2	10.8	7	--	88	2.5	1020	5.2	8.8
	23	11.1	0	81	4.6	1015.5	8	10.7	6.3	--	88	3	1019.9	4.5	8.4
	24	10.9	0	81	3.4	1015.2	7.8	10.6	5.9	--	89	2.3	1019.6	4.2	8.3
May 6	1	10.5	0	82	2.8	1015.1	7.6	10.4	5.7	--	90	2.1	1019	4.2	8.2
	2	10.4	0	83	3.6	1014.6	7.6	10.5	6.4	--	90	2.1	1019	4.9	8.7
	3	10.3	0	83	3.4	1014.9	7.6	10.4	6.2	--	90	1.2	1019.2	4.7	8.5
	4	10.3	0	83	3.7	1015.1	7.6	10.4	5.6	--	91	1.6	1019.2	4.3	8.3
	5	10.2	0	84	3.4	1015.4	7.6	10.5	5.9	--	91	1	1019.1	4.6	8.5
	6	10.5	0	83	2.6	1015.3	7.7	10.5	7.8	--	89	1	1019.1	6.1	9.4
	7	12.1	0	76	3.7	1015.1	8	10.7	11	--	79	2.4	1019.3	7.5	10.4
	8	14.6	0	65	2.7	1014.9	8.2	10.9	12.2	--	72	1.6	1019	7.3	10.2
	9	17.7	0	56	3.4	1014.6	8.6	11.2	13.8	--	70	3.1	1018.8	8.4	11
	10	19.6	0	48	3	1014.3	8.3	10.9	14	--	73	3.7	1018.7	9.3	11.7
	11	21.5	0	44	3.2	1013.7	8.2	10.9	15	--	76	4.1	1018	10.8	13
	12	22.4	0	47	4.3	1012.8	10.5	12.7	15.1	--	72	2.8	1016.8	10.1	12.4
	13	23.2	0	44	2.3	1012.1	10.2	12.4	15.1	--	73	3.7	1016.2	10.3	12.5
	14	22.4	0	44	3.2	1011.8	9.1	11.6	16.3	--	75	3	1015.6	11.9	13.9
	15	22.3	0	43	2.6	1010.9	8.8	11.3	15.3	--	74	2.5	1015	10.7	12.9
	16	21.9	0	52	3.3	1011.2	11.5	13.6	15.8	--	74	1.6	1014.9	11.2	13.3
	17	20.1	0	53	3.9	1011.5	10.3	12.5	16	--	74	0.6	1015.2	11.4	13.5
	18	18.9	0	55	3.9	1011.4	9.7	12	17.7	--	61	2.5	1014.8	10.1	12.4
	19	18.2	0	56	2.9	1011.8	9.6	11.9	16.6	--	63	2.2	1015.1	9.5	11.9
	20	17.3	0	58	3.9	1011.9	9	11.5	16.1	--	60	3.3	1015.2	8.3	11
	21	16.8	0	59	2.9	1012.1	8.8	11.3	15.9	--	57	4.4	1015.3	7.4	10.3
	22	16.2	0	62	1.6	1011.4	8.9	11.4	17.1	--	47	4.7	1014.1	5.7	9.2
	23	15.9	0	62	0.5	1010.3	8.6	11.2	14.6	--	64	3.1	1013.7	7.9	10.6
	24	16.3	0	61	1.6	1009.2	8.8	11.3	14.2	--	66	2.9	1012.9	8	10.7

May 7	1	16.1	0	59	1.4	1008.1	8.1	10.8	13.7	--	69	4.1	1011.4	8.1	10.8
	2	15	0	68	2.4	1007.4	9.2	11.6	13.1	--	71	4.1	1011.1	8	10.7
	3	15.1	0	66	1.7	1007	8.8	11.3	12.9	--	72	4.2	1010.6	8	10.7
	4	15.1	0	64	1.9	1006.6	8.3	10.9	11.9	--	77	4.2	1010.2	8	10.7
	5	15	0	62	1.7	1006.2	7.7	10.5	11.8	--	77	3.1	1009.8	7.9	10.7
	6	14.9	0	63	1.2	1005.8	7.8	10.6	13	--	73	2.7	1009.4	8.3	10.9
	7	16.1	0	59	2.4	1005.6	8.1	10.8	17.1	--	60	2.4	1009.1	9.3	11.7
	8	18.8	0	53	1.4	1005.7	9.3	11.7	21	--	53	2.5	1008.8	11.1	13.2
	9	20	0	53	2.2	1005.5	10.1	12.3	21.6	--	47	1	1008.4	9.8	12.1
	10	21.7	0	46	3.1	1005.5	9.6	11.9	19.2	--	57	4.5	1008.5	10.5	12.7
	11	23.4	0	40	3.2	1005	9.1	11.5	19.2	--	57	2.6	1008.5	10.5	12.7
	12	24	0	42	2.7	1004.9	10	12.2	24.4	--	41	5.4	1007.9	10.3	12.5
	13	22	0	52	2.4	1004.4	11.9	13.9	23	--	47	6.5	1007.1	11.1	13.2
	14	22.8	0	51	3.9	1004	12.3	14.3	24.2	--	44	7.6	1006.9	11.2	13.3
	15	23.8	0	46	4.9	1003.4	11.5	13.6	23.7	--	49	6.2	1006.6	12.4	14.4
	16	23.3	0	47	3.6	1003.4	11.7	13.7	24.1	--	46	6.4	1006.1	11.8	13.8
	17	22.9	0	52	4.4	1003.8	12.5	14.5	21.7	--	56	5.5	1006.2	12.6	14.5
	18	23.1	0	50	2.9	1003.7	12.1	14.1	19	0	78	3.4	1006.4	15.1	17.1
	19	23.1	0	50	3.1	1003.7	12.1	14.1	18.1	0	84	3.3	1006.6	15.4	17.4
	20	20.2	0	67	6.1	1005.3	13.9	15.9	17	2	85	3.8	1007.1	14.5	16.5
	21	17.5	3.5	90	4.6	1006.2	15.9	18	17.2	0	85	4.1	1007.8	14.7	16.7
	22	18.7	0	83	5.8	1005.5	15.8	17.9	17	0	85	5.4	1008.2	14.5	16.5
	23	18.6	0.5	83	5.4	1005.9	15.6	17.7	16.7	--	85	6.1	1008.3	14.2	16.2
	24	18	0	88	4.8	1006.2	16	18.2	16.1	--	85	5.2	1008.5	13.6	15.6

Chapter 3:

COMPARATIVE ANALYSIS BETWEEN BACTERIAL POPULATION STRUCTURE OF SAND DUNES AND DUST AEROSOL IN A KOSA SOURCE AREA, THE TAKLAMAKAN DESERT

3.1 INTRODUCTION

The major source areas of the Kosa event are the Taklamakan Desert, Gobi Desert, and Loess Plateau (Sun et al. 2001; Duce et al. 1980; Iwasaka et al. 1983). Dust storms from Taklamakan desert likely inject dust particles into the upper atmosphere, with the dust particles being transported at high altitudes ranging from 3,000–6,000 m for long distances (Iwasaka et al. 2003; Uno et al. 2004). Some studies reported that bacterial communities in the Kosa source regions are composed of several bacterial species and that some of them were identical to airborne bacterial species detected in Japan (Hua et al. 2007). Dunhuang city,

which is located at the east end of the Tarimu basin (Taklamakan Desert), is an optimal site to study the dynamics and composition of the Kosa with microorganisms released from the Taklamakan Desert (Iwasaka et al. 2009). The atmosphere around the Magao Caves in Dunhuang city has been demonstrated to include many species of bacteria, exhibiting variations depending on the numbers of tourists visiting the caves (Wang et al. 2010). The atmosphere in Dunhuang city has to be examined in an area free of contamination due to human activity. Moreover, the bacterial communities in the sand dunes of Taklamakan Desert also need to be characterized in order to evaluate the sources of the airborne bacterial communities.

In this study, bacterial populations from sand dunes and atmosphere of the Kosa source region (Taklamakan Desert) were investigated to identify and establish the dust-associated bacteria. The aim of this study was to understand the dynamics of airborne bacteria in Dunhuang city during a dust event and to compare the airborne bacterial communities with those in sands collected from the Taklamakan Desert for discussing the source of bacterial populations in atmosphere over dust regions.

3.2 MATERIALS AND METHODS

3.2.1 Sampling of air sample and sand particles

Air sampling was performed in Dunhuang city from 7:00 am UTC on September 10 to 7:00 am UTC on September 12, 2012, when a significant dust event occurred (September 10-12). Dunhuang city is located on the eastern border of the Taklamakan Desert, which is the source of yellow dust traveling towards

Japan. The sampling system was placed on a 10-m high platform (located at the roof of Dunhuang city Meteorological Department campus: 40.21°N, 94.68°E). Air samples (520 L) were collected using sterilized polycarbonate filters (0.22 μm pore size; Whatman, Tokyo, Japan) with a sterilized filter holder using an air pump. For each sample, two filters were used continuously for 12 h; the filters were changed every 12 h. In total, four air samples were obtained during the sampling period from the morning of September 10 to the morning of September 12, which were labeled DhA1 to DhA4. Of the two filters used to collect each sample, one filter was used to determine the abundance of bioaerosols by microscopic observation, and the other one was stored at –80°C before the extraction of genomic DNA for the analysis of bacterial species composition.

The sand samplings were performed at four sampling sites in the Taklamakan Desert (Magao Cave (40.04°N, 94.74°E), Ming Sha Mountain (40.09°N, 94.67°E), Peacock Rock, and Sphinx Rock (40.53°N, 93.06°E): Fig. 3.1) on September 14 and 15, 2012. These samples were named from DhS1 to DhS4, respectively. The sand dune area of the Magao Cave is located 1 km away from any tourist activity. Ming Sha Mountain is located 15 km from Dunhuang city. Peacock Rock and Sphinx Rock were in the Geological National Park (Taklamakan Desert), located 100 km from Dunhuang city. The sand dunes in these two locations are expected to be free of interference due to human activity. In each sampling site, the sand samples were collected directly from the sand dunes using sterilized test tubes. The samples were transported to the laboratory under dark conditions and preserved at -80 °C.

3.2.2. Characteristics and trajectories of air masses

To track the transport pathways of air masses, 72-h backward trajectories were calculated using the National Oceanic and Atmospheric Administration (NOAA) Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model (http://www.arl.noaa.gov/HYSPLIT.php). The position of the backward trajectory start point was used as the air sampling area for this study (40.21°N, 94.68°E), from a site 10 m above the ground level to estimate the accurate trajectories of air masses in the free troposphere.

3.2.3. Microscopic analysis of particle abundance

To determine the particle abundance 1 mL of sterilized water with paraformaldehyde at a final concentration of 1% was added to one of the filter folders to fix the aerosols. After a 1-h incubation period, the filter was stained with 4, 6-diamidino-2-phenylindole (DAPI) at a final concentration of 0.5 μg/mL for 15 min (Porter and Feig 1980). Next, the filter was placed on a slide on a drop of low-fluorescence immersion oil. A second drop of oil was added, and a coverslip was placed on top. The prepared slides were then observed using an epifluorescence microscope (Olympus, Tokyo, Japan) equipped with an ultraviolet excitation system. A filter transect was scanned, and the mineral particles (white particles), yellow particles and bacterial cells on the filter transect were counted. The detection limit of aerosols was below 5 × 103 particles/m3 of air.

3.2.4. Clone libraries of bacterial 16S rDNA

After the sampling, the aerosols were washed off the filters by shaking with 5 ml of sterilized water containing 0.9% (w/v) of NaCl. Mineral particles of

sand samples (5 mg) were suspended into 5 ml of 0.9% NaCl sterilized. Three mL of the solutions of air samples or sand samples were used for the extraction of genomic DNA (gDNA) using SDS, proteinase K, and lysozyme as described previously (Maki et al. 2008). The gDNA was purified by phenol–chloroform extraction, chloroform extraction, and ethanol precipitation. Fragments of 16S rDNA (1,450 bp) were amplified from the extracted gDNA by PCR using the following oligonucleotide primers: 27F, 5′–AGA GTT TGA TCM TGG CTC AG‑3′; and 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 a total of 30 cycles. The PCR amplicons were purified by phenol-chloroform extraction and chloroform extraction, followed by ethanol precipitation.

The PCR amplicons of 16S rDNA fragments were cloned into Escherichia coli HST08 using a commercially prepared vector PCRII-TOPO with a TA Cloning Kit (Invitrogen, CA, USA) according to the manufacturer's protocol using Taq polymerase, M13 forward and reverse primer. More than 50 clones were obtained from each sample. The nucleotide sequences were determined using a Dye DeoxyTM Terminator Cycle Sequencing Kit (ABI, CA, USA) and a DNA autosequencing system (ABI, Model 373A) according to the manufacturer's recommended protocol. The M13 forward and reverse primers were used as the sequencing primer. The defined sequences were compared with DDBJ (DNA Data Bank of Japan) database and a phylogenetic tree was constructed according to the neighbor-joining algorithm using DNASISpro 3.0 software (Saitou and Nei 1987).

3.2.5. Bacterial isolation from air and sand samples

For the isolation of bacteria from air and sand samples, 2.0 ml of the solution of both samples was inoculated into 19.5 ml of TS (Trypticase Soy Peptone) liquid medium (17 g trypticase peptone, 5 g phytone peptone, 2.5 g K2 PO4, and 2.5 g glucose in 1 l of pure water). Microbial growth was estimated every 2 days at 550 nm absorbance. After 12 days of incubation, 10 microliter of the culture was plated on TS agar plate. After the bacteria isolates were incubated for 3 day in 10 ml of TS medium, the bacterial cells were collected using the centrifugation of 20,000 ×g for 5 minutes (Maki et al. 2012). The bacterial cells were extracted and purified as described above. Fragments of 16S rDNA (1,450 bp) were amplified from the extracted gDNA by PCR using primer, 27F and 1492R. Thermal cycling was performed using a Program Temp Control System PC-700 under the same condition as describe above (Maki et al. 2008). The PCR amplicons were purified by phenol-chloroform extraction and chloroform extraction, followed by ethanol precipitation. The nucleotide sequences were determined as describe above, except that the sequence primer was replaced with 27F and 1492R primers. The determined sequences were compared with DDBJ database and phylogenetically analyzed as describe above (Saitou and Nei 1987).

3.2.6 Accession numbers

The DDBJ accession numbers for the 16S rDNA sequences determined in this study are from AB924227 to AB924368 (Table 3.1) and LC055609 to LC055615 (Table 3.2).

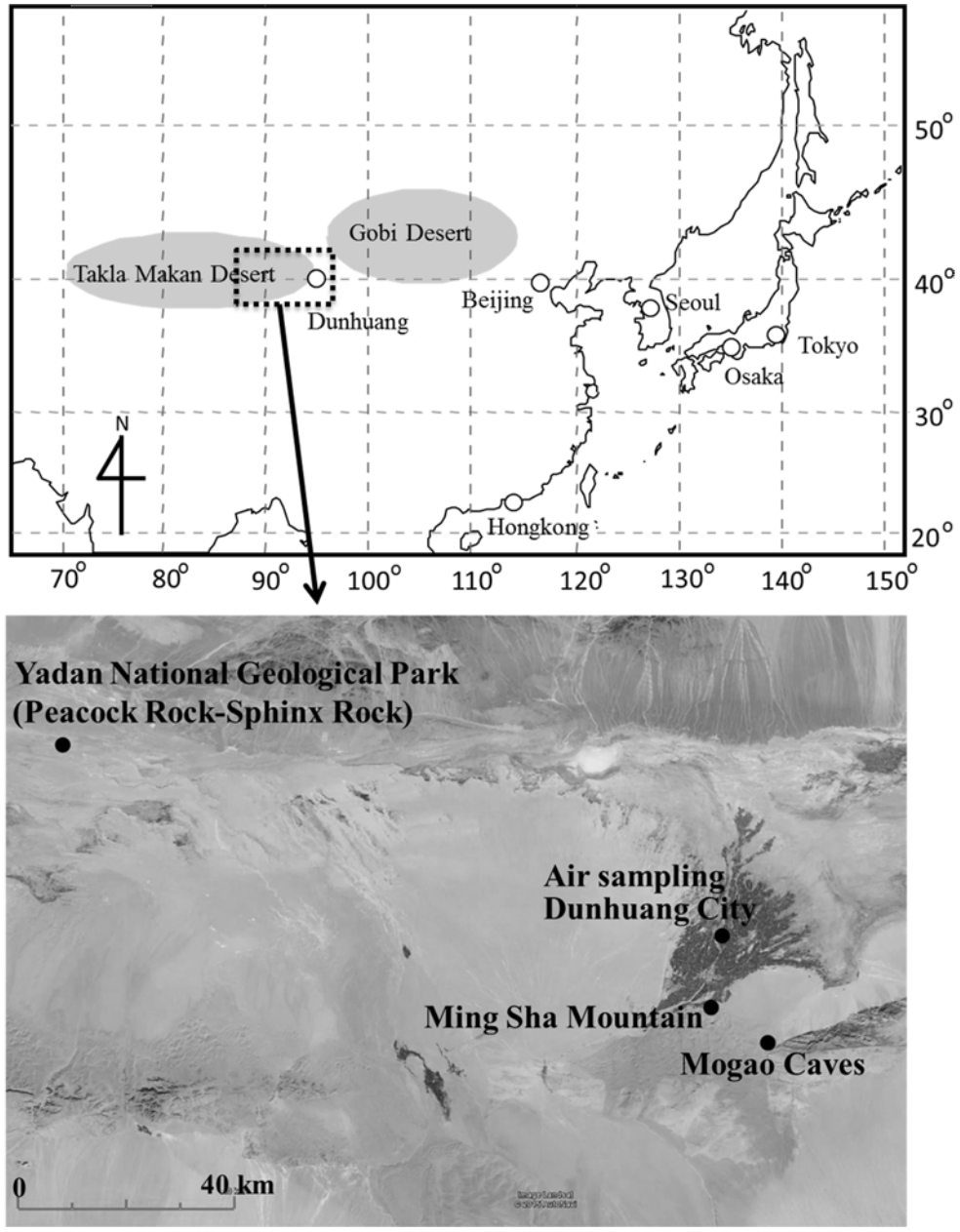


Fig. 3.1 Sampling site (Dunhuang city) and Asian dust (Kosa) source region (Taklamakan Desert)

3.3 RESULT AND DISCUSSION

3.3.1. Transport trajectory and environmental factors

Analysis of air-mass backward trajectory across three days (from 10 to 11 September 2012) indicated that the air mass source was carried from the Taklamakan Desert to the sampling sites at Metrological central department in Dunhuang city (Fig.3.3). During air sampling period, temperature fluctuated between 7 °C and 29 °C and relative humidity ranged from 19% to 73% (Fig 3.2). Air samples were collected on the roof of Dunhuang meteorological department, 10 meters above the ground and on two different days (September 10-11, 2012). Bacterial abundance was determined using microscopic observation after DAPI staining. White-blue fluorescent particles, which were mineral particles, exhibited relatively large sizes with diameters ranging from 0.2 μm to 100 μm and bright-blue fluorescence particles, which were bacteria with smaller diameter <1.0 μm. The concentrations of airborne bacterial cells and white particles increased ten-fold during a dust event when compared to non-dust periods (Fig. 3.4). The abundance of white particles was 10^6 particles/m^3 on the first day, and this increased to over 10^7 particles/m^3 subsequently. This suggested that the dust event occurred during the second day of the sampling period. The bacterial concentration in the atmosphere of Kosa source region maintained a high value of more than 10^6 particles/m^3, subsequently, increasing to more than 10^8 particles/m^3.

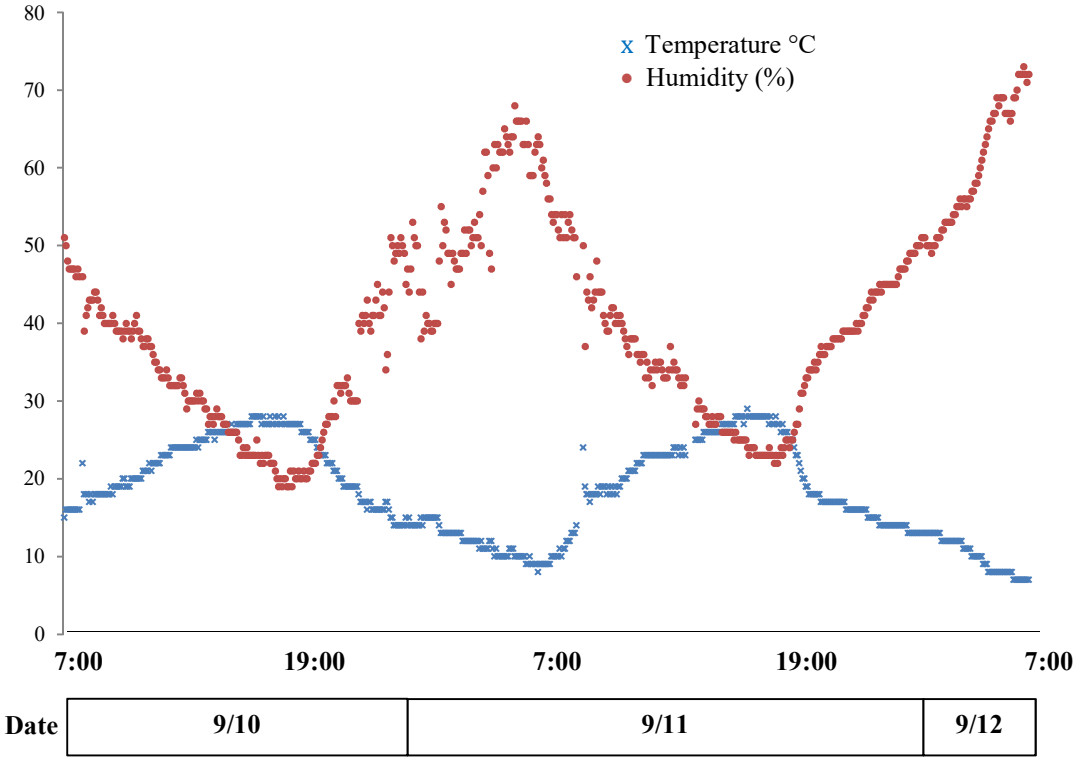


Fig. 3.2 Temperature and relative humidity at Dunhuang city (10-12 September, 2012)

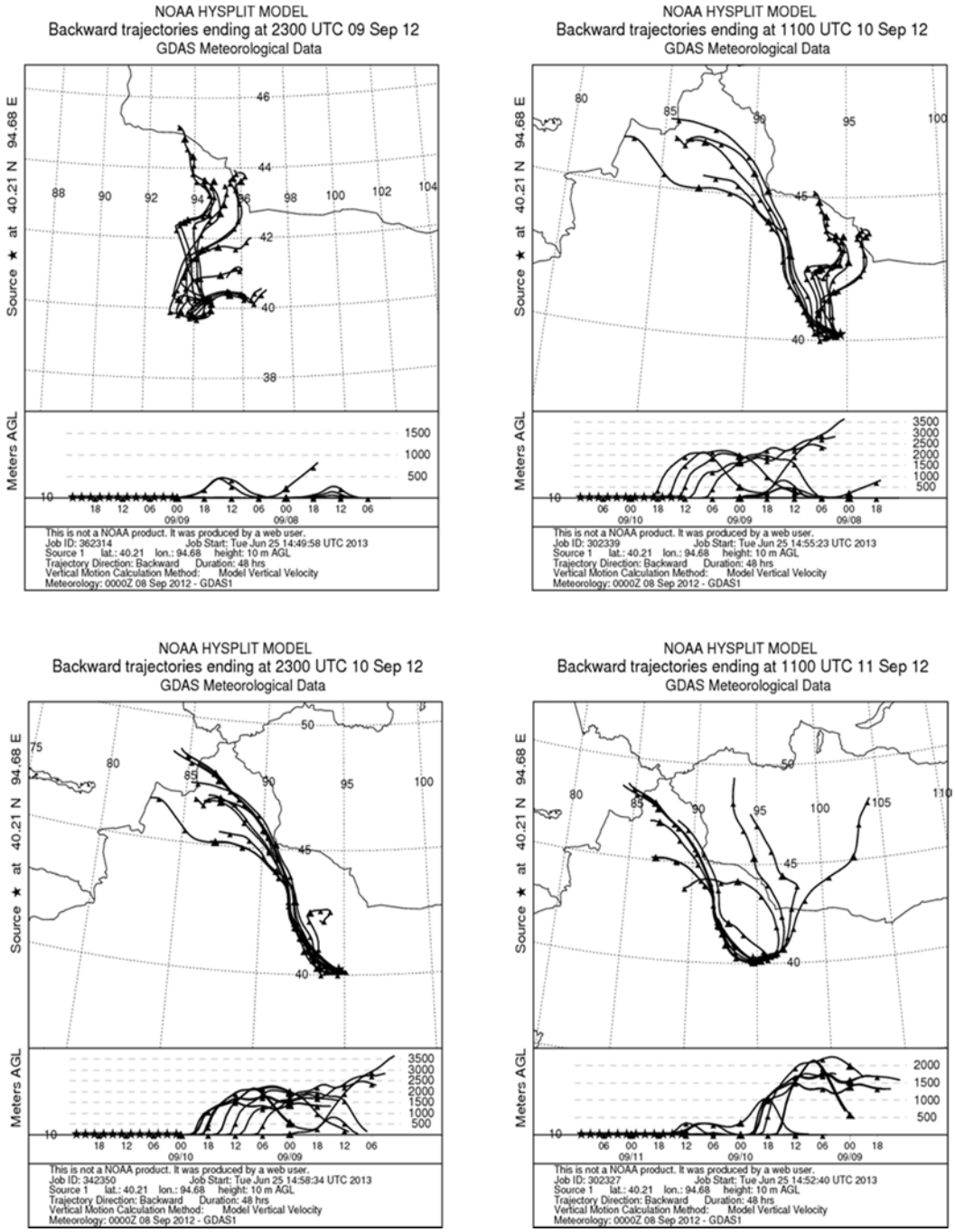


Fig. 3.3 Backward trajectories of air masses containing aerosols at Dunhuang city over three days (September 9-11, 2012).

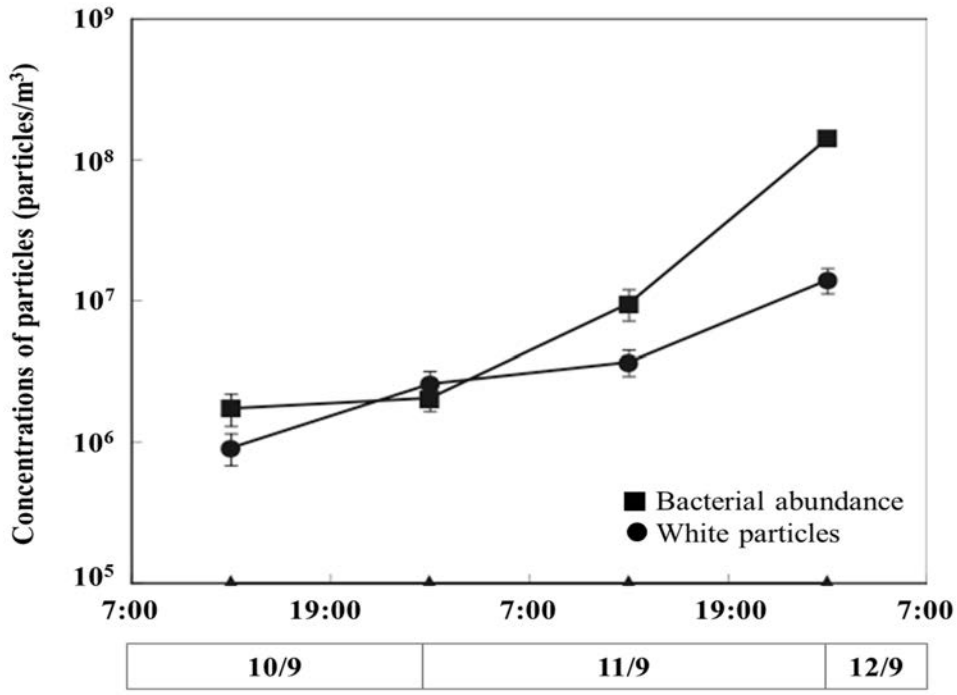


Fig. 3.4 Particle concentrations in the atmosphere 10 m above the ground at Dunhuang city Meteorological Department campus (10-12 September, 2012)

3.3.2. Bacterial composition in air samples

After the genomic DNAs were extracted from the air samples from DhA1 to DhA4, the 16S rDNA fragments of the air samples were amplified using PCR. The PCR amplicons were cloned into *E. coli*, and a total of 386 clones were obtained. The phylogenetic diversity among samples was analyzed using clone libraries of bacterial 16S rDNA. Sequences of the 16S rDNA indicated that the bacterial populations were divided into 46 phylotypes (sequences with >97% similarity; Table 3.1). The bacterial compositions in air-sample clones were composed of similar species among the four samples, and the ratio of clones

indicated bacterial dynamics during the sampling period. Majorities of the phylotypes from the air samples belonged to the phyla *Firmicutes*, *Proteobacteria*, and *Cyanobacteria* (Fig. 3.5).

Bacillus subtilis in *Firmicutes* constituted almost 30% of the clones in DhA3 and DhA4, and this ratio is greater than that during non-dust periods. The isolates of *B. subtilis* were also collected from the air samples and identified with the clone sequences (Fig. 3.6). These results suggested that *B. subtilis* was transported with the dust event. *B. subtilis* can form resistant endospores that enhance their survival in the atmosphere and improve resistance to environmental stressors such as heat, UV, and extreme desiccation (Nicholson et al. 2000). The *Firmicutes* group, including *Bacillus* sp., was reported to be predominantly present in the air just above the ground during Kosa events (Jeon et al. 2011). Some halotolerant bacteria isolated from sand dunes in the Gobi Desert belonging to the genus *Bacillus*, including species were isolated in Higashi-Hiroshima, Japan, indicating the possibility of their long-range transport (Hua et al. 2007). In the air samples DhA1 and DhA2 obtained during non-dust periods, alpha proteobacterial populations in *Proteobacteria,* and unidentified *Cyanobacteria* constituted more than 40% of the clones and this ratio halved in non-dust periods. *Proteobacteria* and *Cyanobacteria* have been reported to be plant associated bacterial (Hu et al 2001; Fürnkranz et al. 2008) and the most common phylum in the high saline environments such as marine (abundance between 50% and 80%) (Desriac et al. 2013) and the terrestrial environments (Janssen 2006; Spain et al. 2009). These results suggested that the major airborne bacteria during non dust events originated from the environment surrounding the sampling site.

Some clones of the *Firmicutes* were related with *Clostridium* species and detected from only the air samples. Commensal *Clostridia* are gram-positive, rod-shaped bacteria in the phylum *Firmicutes* and makeup a substantial part of the total bacteria in the gut microbiota (Bryant 1959; Lopetuso et al. 2008; Nagano et al. 2012). Some *Clostridium* clusters are predominant in the gut microbiota (Hold et al. 2002). The members of *Clostridium* detected from the air samples over Dunhuang city are proposed to have originated from the gut of the animals that live around the sampling sites. Furthermore, other clones of *Firmicutes* in air samples are related to *Staphylococcus* species. *Staphylococcus* strains of normal human flora have been found in and around swimming pools and similar environments (Okafor 2010). *S. aureus* and *S. epidermidis* colonize a sizable portion of the human population (Gill et al. 2005). The staphylococci are ubiquitous colonizers of human or animal skin and mucous membranes, causing a variety of syndromes (Ito et al. 2003). The members of the genus *Staphylococcus* are proposed to have originated from the human activating environment of Dunhuang city.

In addition to *Firmicutes*, clones of the phylum *Proteobacteria* were commonly detected in air samples relating to the members of *alpha proteobacteria*, which are known as freshwater bacteria (Zwart et al. 2002) and oligotrophic pelagic bacteria (Morris 2002). The extensively branching clade of abundant, globally distributed aquatic alpha proteobacteria is adapted to nutrient-poor environments such as the surface waters of the open ocean (Tripp 2013). The rivers around Dunhuang city and the saline-water pools in desert area would be sources of airborne bacterial populations. Moreover, some *Proteobacteria* clones

in the air samples were related with *Stenotrophomonas* sp.. *Stenotrophomonas maltophilia* is ubiquitous in the environment; it has been recovered from the water, soil, plants, animal sources, and sewage (Senol 2004; Berg et al. 1999). This bacterial population of *Stenotrophomonas* sp. is a part of the surrounding environment of Dunhuang city.

3.3.3. Bacterial composition in sand samples

The phylogenetic diversities among sand samples were also analyzed using 16S rDNA clone libraries. Sequences of 196 clones recovered from the sand samples were divided into 24 phylotypes (sequences with >97% similarity; Table 3.1). The bacterial species composition is not significantly different across the four sand samples at Taklamakan Desert (Magao Cave, Ming Sha Mountain, Peacock Rock, and Sphinx Rock). Majorities of the phylotypes from sand samples were composed of the member of the phyla *Actinobacteria*, *Firmicutes,* and *Proteobacteria*. The most dominant bacterial species in sand samples were *Propionibacterium* sp. in the *Actinobacteria*, followed by *B. subtilis* of *Firmicutes,* and *Stenoxybacter* sp. of the *Proteobacteria* (found specifically in deserts). In the previous reports, the most common phyla present in the soil samples collected in Taklamakan and Gobi Deserts were *Firmicutes, Proteobacteria, Bacteroidetes,* and *Actinobacteria* (An et al. 2013) and in the other continental desert, such as Tataoune (Chanal et al. 2006). In the Loess plateau where high amounts of dust particles were accumulated and become to soils, the most abundantly found phyla were *Proteobacteria*, followed by *Bacteroidetes*, *Gemmatimonadetes*, *Actinobacteria*, *Cyanobacteria*, *Chloroflexi,*

Firmicutes, *Chlamydiae*, and *Nitrospirae* (Kenzaka et al. 2010). The members of *Alphaproteobacteria*, *Acidobacteria,* and *Actinobacteria* were also often abundant in soil and members of *Bacteroidetes*, *Firmicutes*, and *Planctomycetes* are generally less abundant (Janssen 2006; Kim et al. 2010). Some bacterial species that were previously reported in samples from the desert areas were not overlapped to the four sand samples in this study. This could be attributed to variations in environmental conditions in desert areas and different methods used to estimate bacterial diversity.

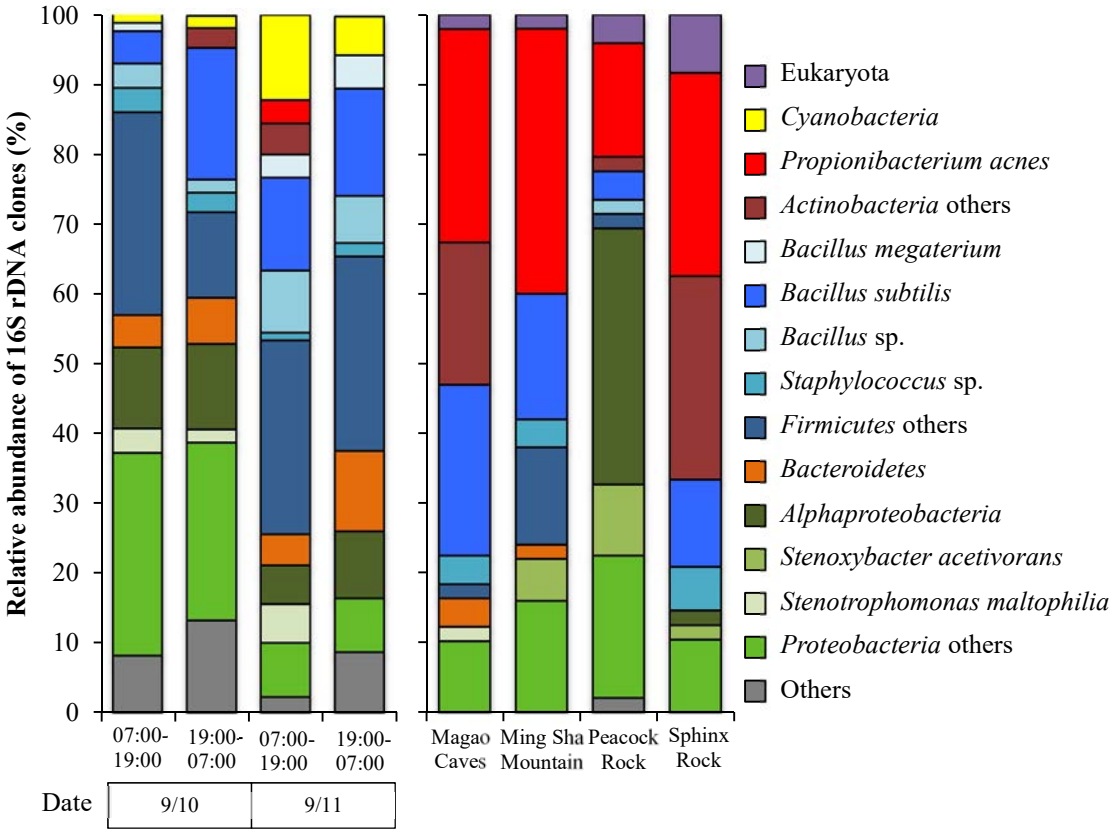


Fig. 3.5 Composition of bacterial species in sand and air samples based on clone libraries of bacterial 16S rDNA

Table 3.1 Phylogenetic affiliation of 16 rRNA gene sequences obtained from clone libraries

Category	Clone No.*1	Number of clones*2	Sampling site*3	Length (bp)	GenBank accession no.	Closest relative	Similarity*4 (%)
Air samples							
Actinobacteria	DhA3-51	3	A3	600	AB924228	*Propionibacterium acnes* isolate WD1 (AY642054)	100
	DhA2-64	1	A2	570	AB924313	*Arthrobacter* sp. EP04 (AM398213)	100
Alphaproteobacteria	DhA1-3	10	A1,3,4	650	AB924235	Alpha proteobacterium SCGC AAA288-E22 (HQ675673)	97
	DhA1-52	11	A1,2	630	AB924232	Alpha proteobacterium IS0505 (DQ517187)	91
	DhA3-131	2	A3,1	650	AB924277	*Sphingomonas* sp. (EU332828)	96
	DhA1-32	3	A1,2	630	AB924253	Bacterium WHC1-2 (JQ269270)	96
	DhA2-7	2	A2,4	570	AB924263	Bacterium SH1-7 (JQ269250)	90
	DhA3-113	1	A3	599	AB924280	Bacterium WHC5-1 (JQ269290)	99
Betaproteobacteria	DhA1-46	3	A1,4	630	AB924254	*Acidovorax* sp. (AJ012071)	99
	DhA1-35	2	A1	630	AB924267	Arsenite-oxidizing bacterium NT-5 (AY027498)	99
	DhA1-28	2	A1	630	AB924266	*Limnohabitans* sp.	98
Gammaproteobacteria	DhA1-48	10	A1,2,3	570	AB924233	*Stenotrophomonas maltophilia* (AM743169)	100
	DhA1-15	7	A1	596	AB924239	*Stenotrophomonas* sp. (DQ256392)	100
	DhA1-49	4	A1,3	595	AB924246	*Pseudomonas* sp. (KC871534)	100
	DhA3-138	2	A3	597	AB924261	*Acinetobacter* sp. (GU566334)	100
Proteobacteria	DhA2-11	22	A1,2	630	AB924230	Proteobacterium BHI60-11 (AJ431219)	98
	DhA2-19	8	A1,2	630	AB924238	Proteobacterium BHI60-9 (AJ431217)	98
Bacteroidetes	DhA4-196	3	A2,4	650	AB924262	*Adhaeribacter aerophilus* (GQ421850)	99
	DhA-51	2	A2,1	561	AB924268	*Adhaeribacter* sp. (GU933569)	99
	DhA2-61	2	A2,3	570	AB924272	Bacteroidetes bacterium (KC560021)	98
	DhA4-171	2	A4	650	AB924283	Flexibacteraceae bacterium 1351 (EU543663)	98
Cyanobacteria	DhA4-74	7	A2,3,4	630	AB924241	*Synechococcus* sp. (CP000097)	99
	DhA3-19	6	A2,3	641	AB924243	*Halospirulina* sp. (JX912466)	97
	DhA4-178	5	A3,4	650	AB924247	Unidentified cyanobacterium (AJ428862)	98
Firmicutes	DhA4-116	53	A1,2,3,4	590	AB924227	*Bacillus subtilis* (AB480778)	100
	DhA4-126	15	A2,3,4	650	AB924231	*Bacillus* sp. (AB020193)	100
	DhA3-39	9	A1,3,4	660	AB924236	*Bacillus megaterium* (EU869261)	99
	DhA4-180	7	A1,3,4	650	AB924240	Clostridiales bacterium (AB702937)	96
	DhA4-192	5	A1,3,4	650	AB924242	*Salinicoccus kunmingensis* (DQ837380)	99
	DhA1-5	4	A1,2,3	596	AB924271	*Staphylococcus* sp. (AJ704792)	100
	DhA1-46	4	A1,3	570	AB924248	*Jeotgalicoccus* sp. (DQ358647)	99
	DhA1-51	4	A1	570	AB924249	*Clostridium* sp. (AB739698)	93
	DhA1-42	3	A1,2,3	570	AB924255	*Roseburia hominis* (AB661434)	96
	DhA4-179	3	A1,4	650	AB924256	*Alkalibacterium iburiense* (AB294177)	99
	DhA2-54	2	A1,2	570	AB924270	*Staphylococcus equorum* (EU855190)	100
	DhA2-36	2	A2	570	AB924274	Bacterium ID4395 (EU660427)	98
	DhA3-124	2	A3,4	650	AB924276	*Lactobacillus johnsonii* DPC 6026 (CP002464)	98
	DhA2-63	1	A2	568	AB924321	*Clostridium saccharolyticum* (CP002109)	95
	DhA4-62	1	A4	589	AB924244	*Staphylococcus epidermidis* (JX898022)	100
	DhA1-6	1	A1	710	AB924305	*Bacillus aryabhattai* (KC764988)	100
	DhA2-42	1	A2	567	AB924318	*Bacillus benzoevorans* (Y14693)	99
	DhA3-24	1	A3	440	AB924334	*Bacillus tequilensis* (JX134629)	99
	DhA4-160	1	A4	650	AB924350	*Bacillus humi* (AJ627210)	99
	DhA4-131	1	A4	650	AB924351	*Bacillus licheniformis* (HQ154527)	99
Deinococcus-Thermus	DhA4-72	4	A1,2	829	AB924251	*Deinococcus* sp. (DQ865058)	99
Bacteria	DhA2-9	11	A1	630	AB924234	Marine sponge bacterium PLATEdelici-(3)-6 (EU346576)	98
	DhA1-44	2	A1	630	AB924286	Bacterium 10RO2 (AY928233)	99
Sand samples							
Actinobacteria	DhS4-44	42	S1,2,3,4	600	AB924228	*Propionibacterium acnes* isolate WD1 (AY642054)	100
	DhS1-7	34	S1,2,3,4	597	AB924229	*Propionibacterium* sp. 215(113zx) partial (AM410900)	100
	DhS1-21	6	S1,2,4	599	AB924250	*Propionibacterium acnes* (AB924250)	100
Alphaproteobacteria	DhS3-28	6	S3	600	AB924245	Bacterium WH5-5 (JQ269309)	99
	DhS3-36	2	S3	598	AB924290	Bacterium WHC3-9 (JQ269283)	99
	DhS3-25	2	S3	600	AB924292	*Sphingomonas paucimobilis* isolate EPA 505 (U37341)	100
	DhS3-18	1	S3	599	AB924280	Bacterium WHC5-1 (JQ269290)	99
	DhS4-1	1	S4	570	AB924263	Bacterium SH1-7 (JQ269250)	90
	DhS3-47	1	S3	598	AB924366	Alpha proteobacterium SCGC AAA300-M14 (HQ675338)	99
	DhS3-49	1	S3	600	AB924365	Alpha proteobacterium SCGC AAA298-C11 (HQ675217)	98
Betaproteobacteria	DhS2-45	9	S2,3,4	599	AB924237	*Stenoxybacter acetivorans* strain TAM-DN1 (EF212897)	93
	DhS2-43	2	S2	597	AB924287	*Achromobacter* sp. CH1 (HQ619222)	100
Gammaproteobacteria	DhS3-3	4	S3,4	600	AB924293	*Moraxella osloensis* (JX485814)	100
	DhS3-26	1	S3	570	AB924233	*Stenotrophomonas maltophilia* (AM743169)	100
	DhS3-16	1	S3	596	AB924239	*Stenotrophomonas* sp. (DQ256392)	100
	DhS125	1	S1	595	AB924246	*Pseudomonas* sp. (KC871534)	100
	DhS3-5	1	S3	597	AB924261	*Acinetobacter* sp. (GU566334)	100
	DhS3-4	3	S3	598	AB924265	Gamma proteobacterium C0016(2010) (GU947880)	99
	DhS2-24	2	S2	600	AB924288	*Haemophilus parainfluenzae* T3T1	99
Bacteroidetes	DhS1-12	2	S1	599	AB924285	Bacteroidetes bacterium SCGC AAA160-F08 (JF488561)	97
Firmicutes	DhS1-1	32	S1,2,3,4	590	AB924227	*Bacillus subtilis* (AB480778)	100
	DhS4-2	5	S1,2,4	589	AB924244	*Staphylococcus epidermidis* (JX898022)	100
	DhS3-12	1	S3	650	AB924231	*Bacillus* sp. (AB020193)	100
	DhS2-21	1	S2	650	AB924242	*Salinicoccus kunmingensis* (DQ837380)	99
	DhS1-5	1	S1	596	AB924271	*Staphylococcus* sp. (AJ704792)	100
	DhS2-1	1	S2	599	AB924363	*Staphylococcus haemolyticus* strain 33E (KC329826)	100
Eukaryota	DhS4-25	4	S3,4	596	AB924252	*Quercus nigra* (HQ664601)	100

*1 Clones of 16S rDNA library were named as DhS for sand samples and DhA for air samples

*2 The number of the clones in 16 rDNA clone libraries

*3 Sampling period and site when the sample was collected. S1 Magao Cave, S2 Ming Sha Mountain, S3 Peacock Rock and S4 Sphinx Rock; A1 air sample 1, A2 air sample 2, A3 air sample 3 and A4 air sample 4

*4 Similarity value between each isolate and the closest relative in databases

3.3.4. Dominant bacterial species in air and sand samples

Majorities of the phylotypes from the sand and air samples belonged to phyla *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes*. In both sand and air samples, the clones of *B. subtilis* (phylum *Firmicutes*) dominated, occupying more than 10% of the clones. *B. subtilis* was possibly transported from the sand dunes to the atmosphere. Total 9 isolates, which were obtained from the sand and air sample using NaCl-amended culture technique, were closely related or identical to *B. subtilis* which were detected in the air samples DhA series collected in Dunhuang city (97-100% similarity) (Table 3.2, Fig. 3.6).

Table 3.2 Phylogenetic affiliation of sequences of bacterial isolates obtained from sand and air sample

Isolate names	Sampling site	Closest relative	Category	Similarity (%)*)	Length (bp)	GenBank accession no.
DhSi-1	Taklamakan Desert	*Bacillus subtilis* (LN556364)	*Firmicutes*	99	675	LC055609
DhSi-2	Taklamakan Desert	*Bacillus subtilis* (KF687050)	*Firmicutes*	97	1406	LC055610
DhSi-7	Ming Sha Mountain	*Bacillus subtilis* (KM497438)	*Firmicutes*	100	1401	LC055611
DhSi-8	Mogao Caves	*Bacillus subtilis* (KJ875754)	*Firmicutes*	99	1401	LC055612
DhSi-11	Ya Dan	*Bacillus subtilis* (KM497438)	*Firmicutes*	98	1407	LC055613
DhAi-18	Dunhuang 10 m	*Bacillus sonorensis* (KP165033)	*Firmicutes*	99	1338	LC055614
DhAi-39	Dunhuang 10 m	*Bacillus subtilis* (KP792773)	*Firmicutes*	99	841	LC055615

*) Similarity value between each sequence and the closest relative in databases

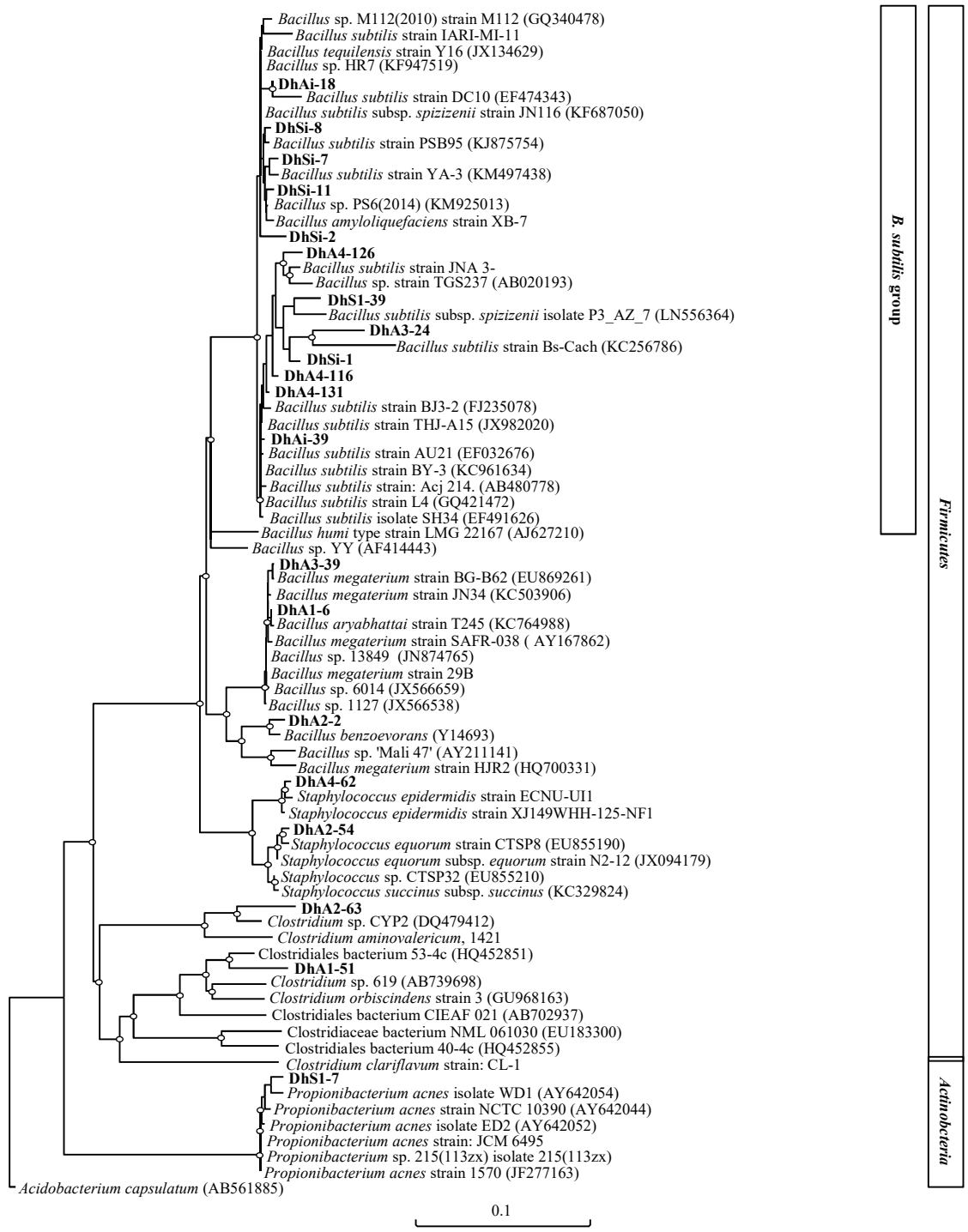


Fig. 3.6 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained from the isolate (sand; DhSi series, air; DhAi series), and the clone libraries from sand (DhS series) and air samples (DhA series) of the *Firmicutes* and *Actinobacteria*. The sample information and the accession number of each reference sequence are given in parentheses. Open circles at branch points show that the bootstrap values obtained by neighbor-joining analysis exceeded 50% (after 1000 resamplings)

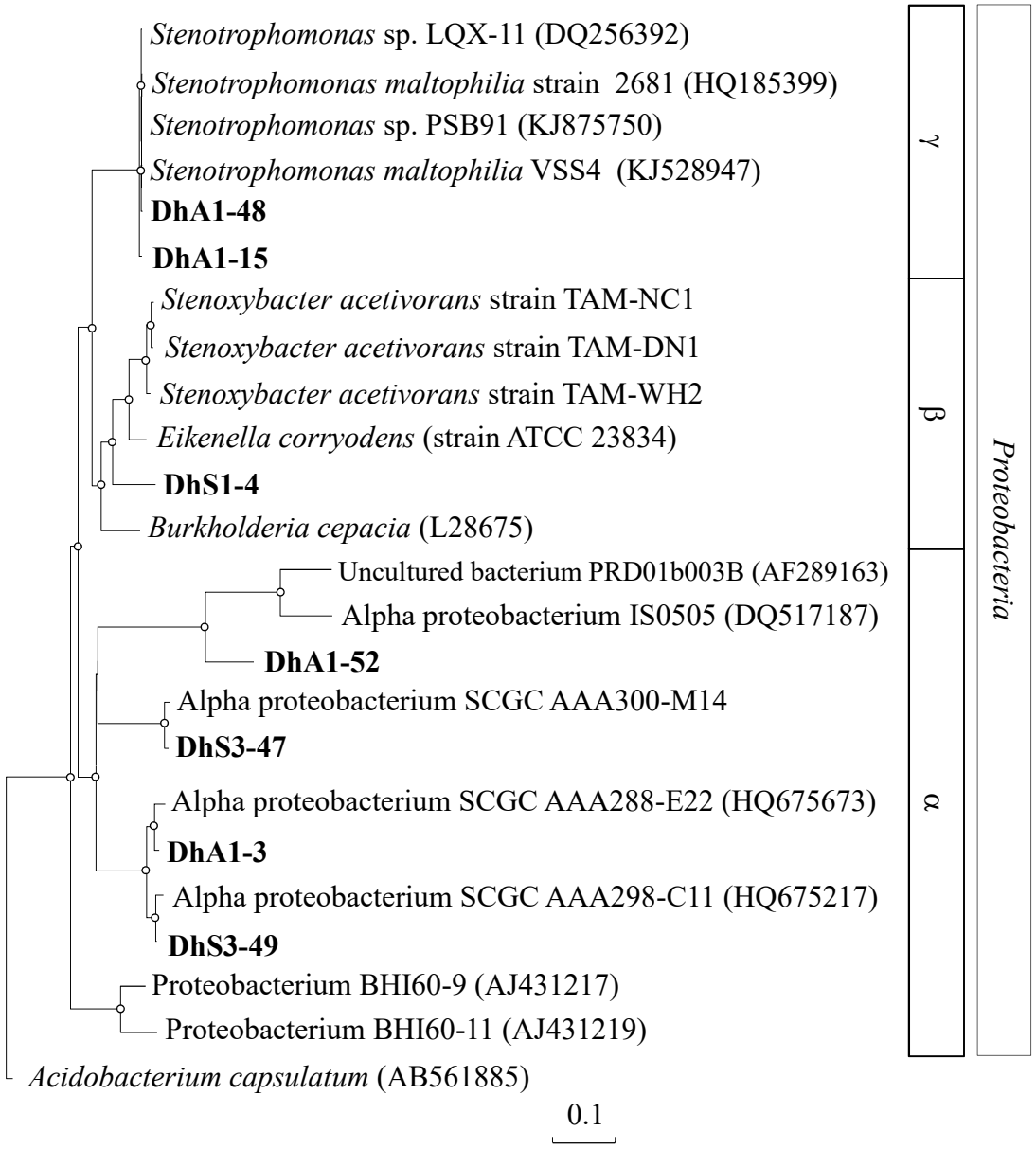


Fig. 3.7 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained from the clone libraries from sand (DhS series) and air samples (DhA series) representative of the *Proteobacteria*. The sample information and the accession number of each reference sequence are given in parentheses. Open circles at branch points show that bootstrap values obtained by neighbor-joining analysis exceeded 50% (after 1000 resamplings)

Chapter 4:

MIXTURE OF AIRBORNE MICROBIAL TO HIGH ALTITUDES OVER KOSA SOURCE AREA, DUNHUANG CITY CHINA

4.1 INTRODUCTION

Sand dune and air mass in the Chinese desert area included several kinds of microorganisms (Hua et al. 2007; An et al. 2013), which are potentially transported for long-range transport to downwind area by Asian dust event. In fact, in downwind area such as Japan (Maki et al. 2014) and Korea (Jeon et al. 2011), the terrestrial bacterial population predominately including *Bacillus* species increased in the correspondence to Asian dust events. The bioaerosols over Kosa source regions has been also analyzed using PCR-denaturing gradient gel electrophoresis (DGGE) analysis (Maki et al. 2008), preliminarily suggesting the vertical mixture processes of dominate bacterial population.

Recent technology, such as massive parallel sequencing, that can analyses large numbers of nucleotide sequences has been applied for estimating the community structures of airborne bacteria on mountains (Bowers et al. 2012) and in rural and urban areas (Brodie et al. 2007) at the family level. Few researches investigated the bacterial communities at high altitudes above ground level have using pyrosequencing analysis (Bowers et al. 2009, DeLeon-Rodriguez et al. 2013), and the vertical distribution of airborne bacteria between the ground and high altitudes over Asian dust source region is unclear. However, the microbial communities in atmosphere over dust source regions have not investigated using massive parallel sequencing technique. These enough amounts of sequence data are expected to provide important suggestions for the vertical mixtures of bioaerosols over Kosa region.

Moreover, the previous bioaerosol survey at high altitudes over Kosa source region focused on only bacteria, and neglected airborne fungal communities. Fungi such as *Bjerkandera adusta* isolated at high altitudes have been reported to induce allergy levels similar to that caused by Asian mineral dust particles (Liu et al. 2014) and fungal population associated with Kosa mineral particles included several kinds of pathogens for human, plants and animals (Chao et al. 2011). Accordingly, fungal population in bioaerosols at high altitudes over Kosa source region should be analysed using massive parallel sequencing.

For more comprehensive understanding of airborne bacterial communities at high altitudes in Kosa source, we collected air samples at altitudes of 800 m by using a balloon and at 10 m from a building, over the east edge of Taklamakan desert (Dunhuang city), China (Fig. 3.1). We analyzed the meteorological data for

estimating the air mass conditions during the sampling periods, and determined the aerosol amounts by using meteorological monitoring techniques. Microbial community structures in the air samples were analyzed using massive parallel sequencing, which depend on synthesis with reversible terminators and targeti bacterial 16S rRNA genes (16S rDNA) and fungal Internal Transcribed Spacer (ITS) regions. However, massive parallel sequencing generates short-length sequences that can lead to misreads, allowing us to understand bacterial composition at the class or family level, but not at the species level (Womack et 2010). Therefore, the compositions of microbial species in the air samples were phylogenetically analyzed using the cloning library technique targeting 16S rDNA and ITS region sequences.

4.2 MATERALS AND METHODS

4.2.1 Air sampling

Air sampling was performed in Dunhuang City Meteorological Department; Dunhuang City is located on the eastern border of the Taklamakan Desert, which is the source of Kosa traveling towards Japan. Sampling was performing on September 10, 2013. The sampling system was placed on 10 m, and 800 m high platform (40.22°N, 94.6°E). 10 m sampled were collected in the roof of Dunhuang city Meteorological Department campus building, and the 800 m samples were collected using a balloon. Air samples (520 L) were collected using sterilized polycarbonate filters (0.22 μm pore size; Whatman, Tokyo, Japan) with a sterilized filter holder using an air pump. For each sample, two filters were used continuously for 1 h. In total, four air samples were obtained, which were

labeled 13Dh10 for 10 m altitude and 13Dh800 for 800 m altitude. Within 1 hour of sampling, the aerosols on the filter were shacked in 10 ml of sterilized water at the concentration of 0.9% (w/v) NaCl. The wash solution was used for the epifluorescent microscopic observation of aerosol particles and for the determination of bacterial species composition by pyrosequencing analysis and TA cloning analysis.

4.2.2 Characteristic and trajectory of air masses

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 position of the backward trajectory start point was used as the sampling area for this study (40.22°N, 94.68°E), from 10 m and 800 m above the ground level to estimate the accurate trajectories of air masses in the free troposphere.

4.2.3 Microscopic analysis of particle abundance

The filter wash solution (1 mL) was fixed with a paraformaldehyde at a final concentration of 1%. After a 1-h incubation period, the filter was stained with 4, 6-diamidino-2-phenylindole (DAPI) at a final concentration of 0.5 μg/mL for 15 min (Porter and Feig 1980). Next, the filter was placed on a slide on a drop of low-fluorescence immersion oil. A second drop of oil was added, and a coverslip was placed on top. The prepared slides were then observed using an epifluorescence microscope (Olympus, Tokyo, Japan) equipped with an ultraviolet excitation system. A filter transect was scanned, and the mineral particles (white

particles), yellow particles and bacterial cells on the filter transect were counted. The detection limit of aerosols was below 5 × 103 particles/m3 of air.

4.2.4 Clone libraries of bacterial 16S rDNA and fungal 18S rDNA

Particles in the filter wash solution (3 mL) of air samples collected at 10 m and 800 m were pelleted by centrifugation at 20,000 × g for 5 min and were used for the extraction of genomic DNA (gDNA) using SDS, proteinase K, and lysozyme as described previously (Maki et al. 2008). The gDNA was purified by phenol–chloroform extraction, chloroform extraction, and ethanol precipitation. Fragments of 16S rDNA (1,450 bp) were amplified from the extracted gDNA by PCR using the following oligonucleotide primers: 27F, 5′–AGA GTT TGA TCM TGG CTC AG–3′; and 1492R, 5′–GGY TAC CTT GTT ACG ACT T–3′ (Maidak et al. 1997) and for 18S rDNA using primers: F1,5′–TGG TTG ATC CTG CCA GAG G-3′; and R1,5′–GGC TAC CTT GTT ACG ACT T–3′. 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 a total of 30 cycles. The PCR amplicons were purified by phenol-chloroform extraction and chloroform extraction, followed by ethanol precipitation.

The PCR amplicons of 16S rDNA and 18S rDNA fragments were cloned into Escherichia coli using a commercially prepared vector with a TA Cloning Kit (Invitrogen, CA, USA) according to the manufacturer’s protocol. More than 50 clones were obtained, and the sequences were determined as described above, except that the sequencing primer was replaced with the M13 forward primer. The

defined sequences were compared with DDBJ database and phylogenetically analyzed (Saitou and Nei 1987).

4.2.5 Analysis of bacterial community structures using pyrosequencing analysis targeting 16S and 18S rDNA sequences

For determining bacterial community composition in the air samples collected 800 m and 10 m, the genomic DNA (gDNA) extracted from the samples were analyzed using pyrosequencing, which facilitates multiplexed partial sequencing of 16S rDNA and 18S rDNA. Fragments of 16S rDNA (approximately 500 bp) were amplified from the extracted gDNA by PCR using universal 16S rDNA bacterial primers 515F,5′- Seq A - TGT GCC AGC MGC CGC GGT AA -3′ and 806R,5′- Seq B - GGA CTA CHV GGG TWT CTA AT -3′, where Seq A and Seq B represent nucleotide sequences targeted by the second PCR primers. The sequences of PCR amplicaon covered the variable regions V4 of the 16S rRNA gene region. For the analysis of fugal communities, fungal ITS primers ITS1-F - KYO1,5′- Seq A - CTH GGT CAT TTA GAG GAA STA A -3′ and ITS2- KYO2, 5′- Seq B - TTY RCT RCG TTC TTC ATC -3′. Thermal cycling was performed using a thermocycler (Program Temp Control System PC-700; ASTEC, Fukuoka, Japan) under the following conditions: denaturation at 94°C for 1 min, annealing at 52°C for 2 min, and extension at 72°C for 2 min for 20 cycles. Fragments of targeted sequences in PCR products were amplified again using the second PCR forward primers (5′- Adaptor C - xxxxxxxx - Seq A -3′) and reverse primer (5′- Adaptor D - Seq B -3′) where Adaptors C and D were used for pyrosequencing reaction. The xxxxxxxx included eight nucleotide sequences tags designed for

sample identification barcoding. Thermal cycling was performed under the following conditions: denaturation at 94°C for 1 min, annealing at 59°C for 2 min, and extension at 72°C for 2 min for 12 cycles. PCR amplicons were purified using the MonoFas DNA purification kit (GL Sciences, Tokyo, Japan). PCR amplicons from each sample were pooled at approximately equal amounts into a single tube for pyrosequencing on Genome Sequencer MiSeq (Illumina, CA, USA) machine. The sequences obtained for each sample were grouped depending on the tag, and, after remove of the tags, average 450 bp of read length were obtained. Negative controls (no template and template from unused filters) were prepared in all steps of the process from DNA extraction to check for contamination.

Before the analysis of bacterial community structures, we removed sequences that were <200 bp in length, those with a phred-equivalent quality score of <25, those containing ambiguous characters, those with an uncorrectable barcode, or those without the primer sequence. The remaining sequences were clustered into phylotypes using QIIME (ver. 1.8.0) with a minimum coverage of 99% and a minimum identity of 97%. The bacterial compositions of phylotypes were analyzed by comparing against the DNA Data Bank of Japan (DDBJ) using BLAST.

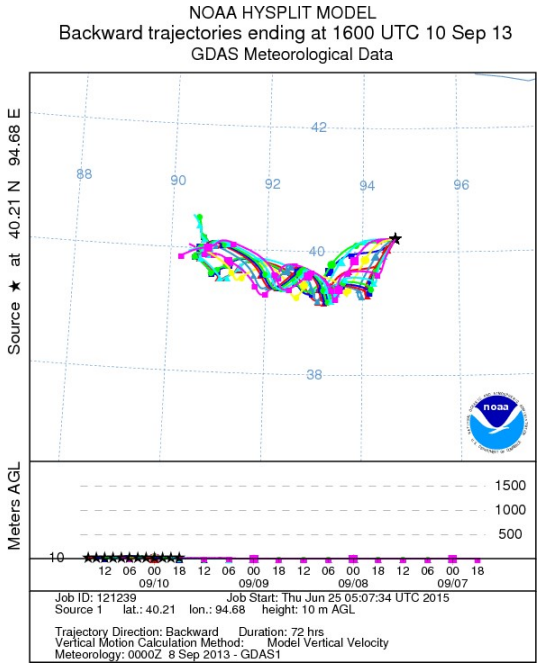
4.3 RESULT AND DISCUSSION

4.3.1 Transport trajectory and environmental factors

Analysis of air-mass backward trajectory indicated that the air mass source was carried from the Taklamakan desert to the sampling sites at Metrological Central Department in Dunhuang city (Fig.4.1). Air samples were collected on the

Dunhuang City Meteorological Department, 10 m and 800 m above the ground on September 10, 2013.

10 m



800 m

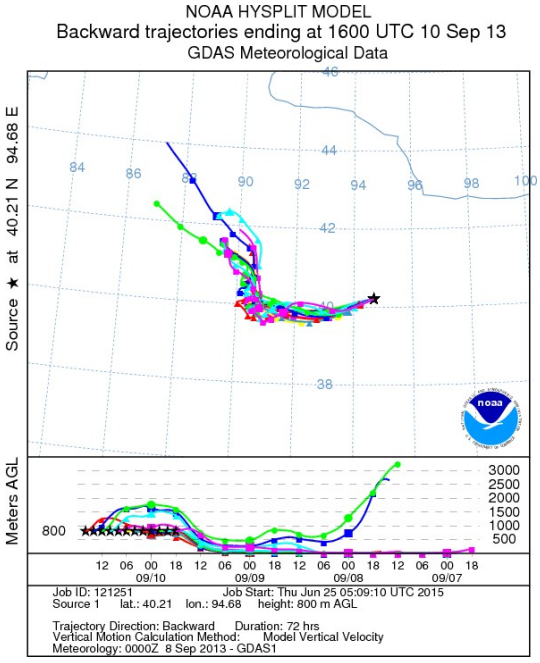


Fig 4.1 Three days backward trajectories of air masses containing aerosols at Dunhuang City September 10, 2013

Bacterial abundance was determined by microscopic observation after DAPI staining. The concentrations of airborne bacterial cells and white particles at altitude 10 m slightly higher than at altitude 800 m (Fig. 4.2). DAPI-stained in both samples included substantial concentrations of mineral particles (white particles), yellow fluorescent particles (organic particles) and microbial particles as observed by epifluorescence microscopy. The 10 m and 800 m sample included mineral particles at concentrations of $(6.82 \pm 3.48) \times 10^5$ particles/m^3 and $(3.79 \pm 1.78) \times 10^5$ particles/m^3, respectively. Yellow fluorescent particles were detected at concentrations of $(0.39 \pm 5.11) \times 10^5$ particles/m^3 and $(0.57 \pm 1.07) \times 10^5$ particles/m^3, respectively. The total density of bacterial cells associated with aerosol particles was $(16.6 \pm 1.19) \times 10^5$ particles/m^3 and $(20.8 \pm 9.15) \times 10^4$ particles/m^3, respectively (Fig 4.2). DAPI yellow fluorescing particles have been reported to resemble organic materials such as protein that originating from microbial cell components (Mostajir et al. 1995).

4.3.2 Bacterial community structures in air sample among two altitudes

Pyrosequencing and clone library databases have been obtained from the air samples collected at two altitudes (10 m and 800 m) above the ground in Dunhuang city. Bacteria diversity in 10 m samples is higher than in altitude 800 m, but the bacterial compositions were similar between the two altitudes, suggesting the vertical mixture of bioaerosols over Dunhuang city. This result agrees with the previous investigations in Dunhunag city showing that the similar DGGE bands appeared among the both altitudes of 10 m and 600 m (Maki et al. 2008).

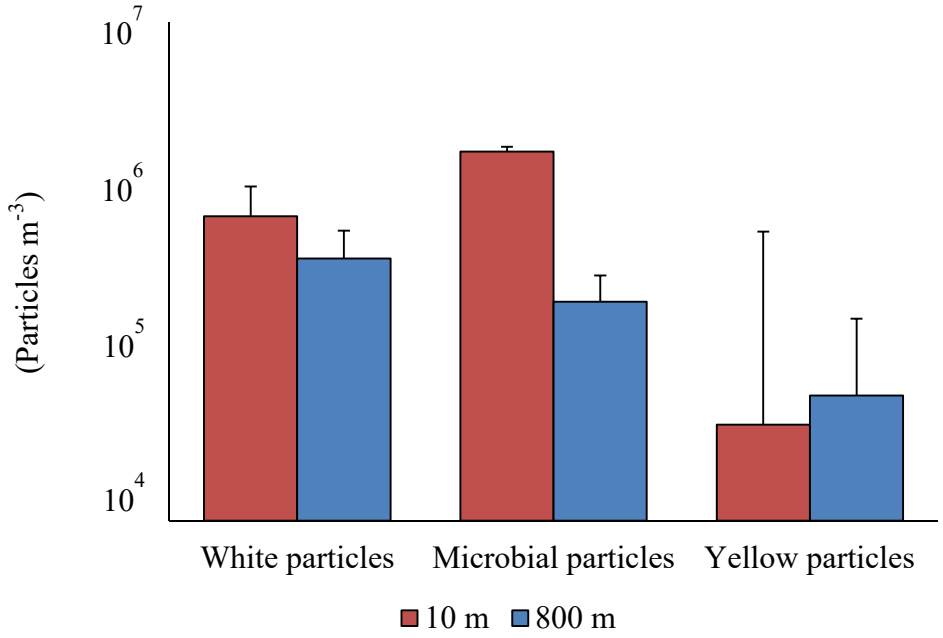


Fig 4.2 Particle concentrations in the atmosphere 10 m and 800 m above the ground at Dunhuang City Meteorological Department Campus September 10, 2013

Total 550,370 taxa were obtained by pyrosequencing analysis of gDNA extracted from four air sample samples (10 m and 800 m), and clustered into 18 phyla (Fig. 4.3). The bacterial communities in the both air samples were predominately composed of the members of phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* (Fig.4.3). *Proteobacteria* is known to be the most abundance phyla in natural environments such as desert and terrestrial area (Janssen 2006; Spain et al. 2009) aquatic field and plant surfaces (Hua et al 2001; Fürnkranz et al. 2008). Clone library analysis demonstrated that the phylum *Firmicutes* is dominated by *Bacillus* species (Fig 4.4). Members of the genus *Bacillus* are known to form endospores that are resistant to environmental stressors to enhance their survival in the atmosphere (Nicholson et al. 2000). The proportions of *Proteobacteria* and *Firmicutes* sequences increased in the samples

of 800 m and decreased in those of 10 m. Contrary, the *Actinobacteria* and *Bacteroidetes* sequences indicated higher proportions in the samples of 10 m than those of 800 m. *Actinobacteria* is soil bacteria and one of the dominant bacterial phyla in soil (Janssen 2006). Yamaguchi et al. (2012) reported that the sequences collected in the soil of the Taklamakan Desert comprised largely of *Bacteroidetes*. These results suggested that the bacteria from the ground level of the Metrological central department seem to have also been included in the sampling and that some of them can remain at high altitudes.

In the phylum *Proteobacteria*, the dominated families in the air samples collected at the both samples were *Xanthomonadaceae* and *Sphingomonadaceae*. The sequences of *Xanthomonadaceae* occupied almost 30% of total sequences in the samples of 10 m and this proportion increase to more than 50% of total sequences in the samples of 800 m. The secondly dominated family is *Sphingomonadaceae,* which occupied about 5% of total sequences (Fig. 4.3). In the phylum *Firmicutes*, the families *Bacillaceae* and *Staphylococcaceae* were dominated in both samples occupying about 20% and 5% of total sequences respectively. *Bacillus* and *Sphingomonas* are predominant bacteria in air sample collected in Mogao Grottoes Dunhuang (Wang et al. 2012), sequence affiliated with *Bacilli* and *Sphingobacteria* are dominated in bacteria carried by Kosa (Yamaguchi et al. 2012). *Bacillus subtilis* in the families *Bacillaceae* were predominant species in the atmosphere above China desert (Maki et al. 2014). *Staphylococcus* are frequently been identified in downwind area during dust event (Griffin et al. 2003).

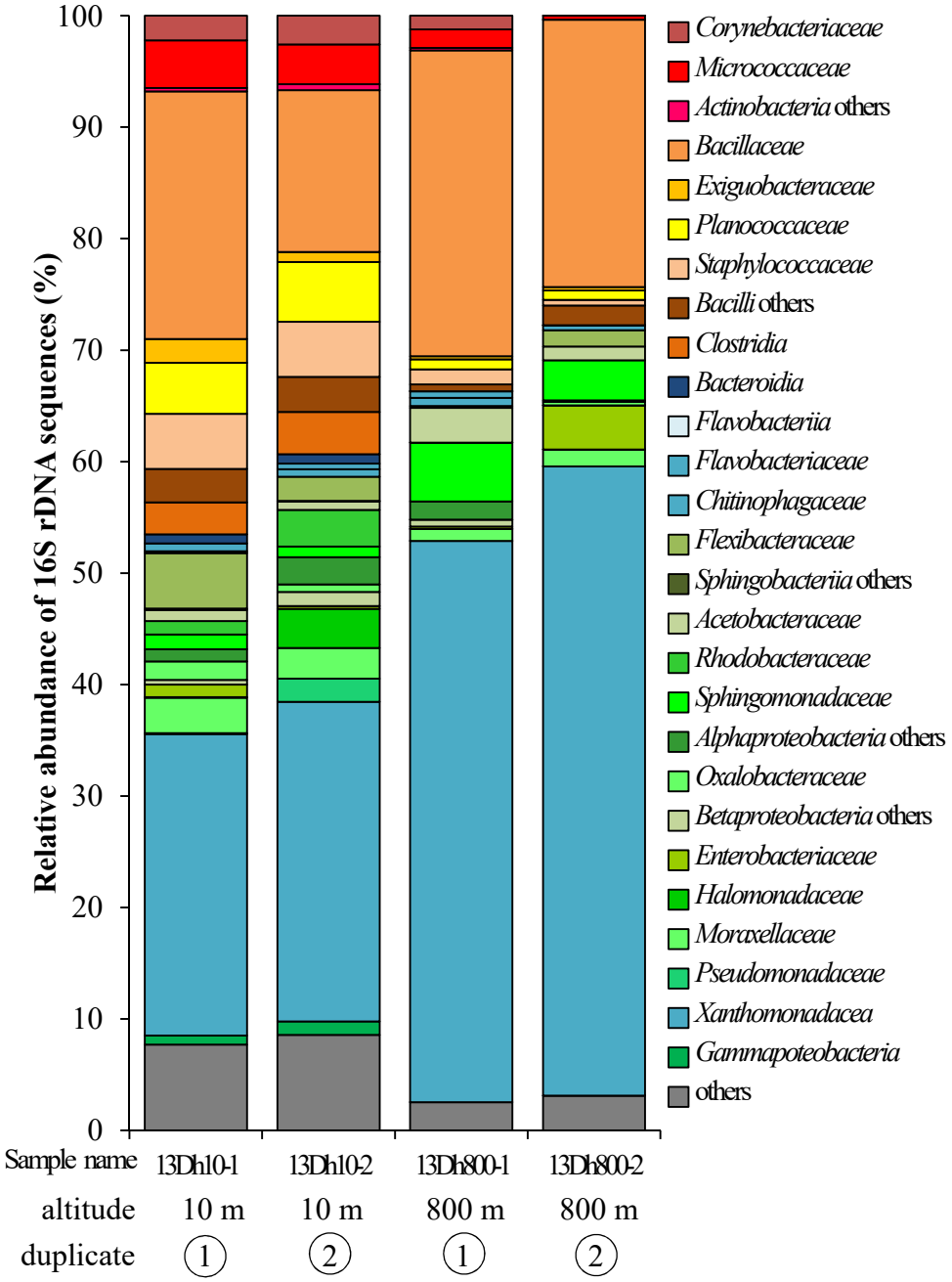


Fig. 4.3 Bacterial compositions at class level of the partial sequences in pyrosequencing database (ca 400 bp) obtained from duplicated air sample (1, 2) collected at altitude of 10 m and 800 m over Dunhuang city on September 10, 2013

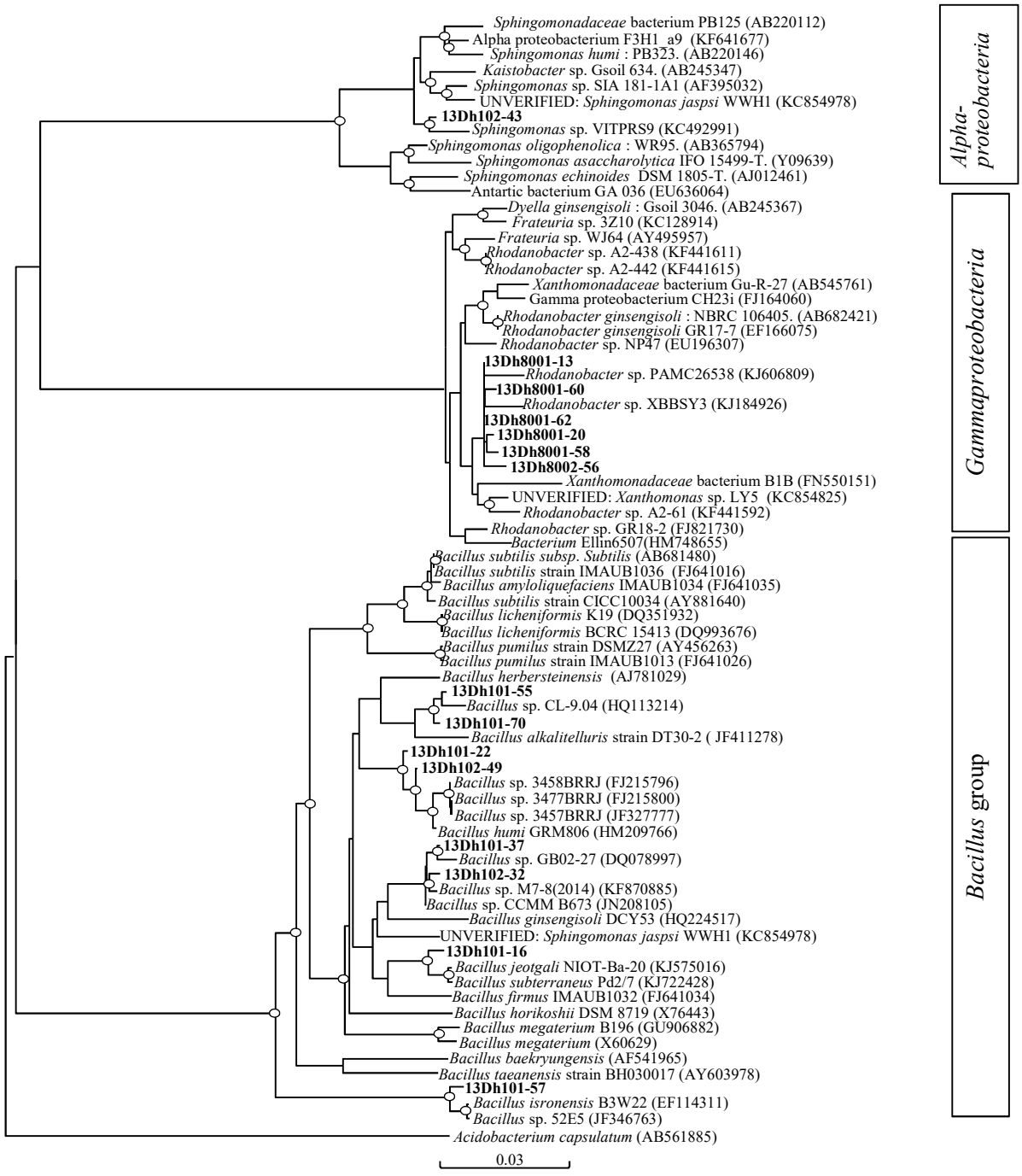


Fig. 4.4 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained from the clone libraries (10 m; 13Dh101, 13Dh102 and 800 m; 13Dh8001, 13Dh8002) from the knowm member of *Firmicutes* and *Proteobacteria*. The sample information and the accession number of each reference sequence are given in parentheses. Open circles at branch points show that bootstrap values obtained by neighbor-joining analysis exceeded 50% (after 1000 resamplings)

4.3.2 Fungal community structures in air samples among two altitudes

In addition to bacterial communities, fungal community structures were also investigated using the pyrosequencing analysis of gDNA extracted from four air samples (10 m and 800 m). Total 68,593 sequences, which were obtained from all samples (10 m; 13Dh10-1, 13Dh10-2 and 800 m; 13Dh800-1, 13Dh800-2), were composed of several fungal taxa varying from 44,713 to 278,520. The unanalyzable sequences such as chimeras, sequences <200 bp and junk sequences, were excluded from the total sequences. Finally, total 34 phylotypes were obtained from all samples and could be clustered into 23 genera. Phylotype numbers of fungi in the sample of 10 m are significantly higher than those of 800 m, suggesting that the diversity of fungal populations reduced in correspondence to higher latitudes (Fig. 4.5).

The fungal compositions in air samples collected at altitude 800 m above the ground in Dunhuang city were dominated by the sequences of only single phylum *Ascomycota*, while those of 10 m were dominated by the members of two phyla *Ascomycota* and *Basidiomycota,* occupying 77% and 23 % of total sequences, respectively (Fig. 4.5) Fungal communities belonging to the phyla *Ascomycota* and *Basidiomycota* are predominantly detected from biospheres (Nowoisky et al. 2008; Solomon et al. 1983). In recent studies, microbial communities in bioaerosols were dominated by the members of the phylum *Ascomycota* than those of the phylum *Basidiomycota* (Despres et al. 2007; Boreson et al. 2004; Fierer et al. 2008). *Ascomycota* species like *Cladosporium* sp and *Penicillium* spp. have small aerodynamic diameters (2-5 μm), while *Basidiomycota* tend to have spores with aerodynamic diameter relatively large (5-

10 μm) (Nowoisky et al. 2008). The fungal spores with smaller diameter are predominant at higher altitude and those of larger diameter more abundant in lower level (Chakraborty et al. 2001). Li et al. (2010) reported the decrease of fungal spore concentrations at higher altitude comparing low altitudes (Li et al. 2010). Accordingly, the members of the phylum *Ascomycota* of which spores are relatively small size would be transported to upper area of atmosphere and predominately appeared at the high altitudes of 800 m.

In the samples of 800 m, the sequences of the genus *Cladosporium* predominately occupied the 98% of total sequences and those of *Penicillium* accounted for the remaining sequences occupying approximately 2% of total sequences (Fig. 4.5). Contrary, the samples of 10 m included the total 34 phylotypes of sequences belonging to phyla *Basidiomycota* and *Ascomycota* (77%, 23% respectively), which were dominated by the members of genera *Cladosporium, Peyronellaea, Alternaria, Fusarium,* and *Cryptococcu* at the abundance ratios of about 15% of the total sequences (Fig. 4.5). Members of the genus *Cladosporium* have been reported to dominate in airborne fungal communities during dust evens in Taiwan (Chao et al. 2011). Moreover, the *Cladosporium* species were commonly detected during Asian and African dust events (Chao et al. 2011; Ho et al. 2005; Wu et al. 2004; Weir-Brush et al. 2004; Griffin et al 2003; Kellogg et al. 2004; Shin et al. 2003; Abdel 2003; Yeo et al. 2002). These results suggested that fungal populations transported from ground surfaces air to atmosphere and that some population such as *Cladosporium* species and *Penicillium* species remain at high altitude of 800 m.

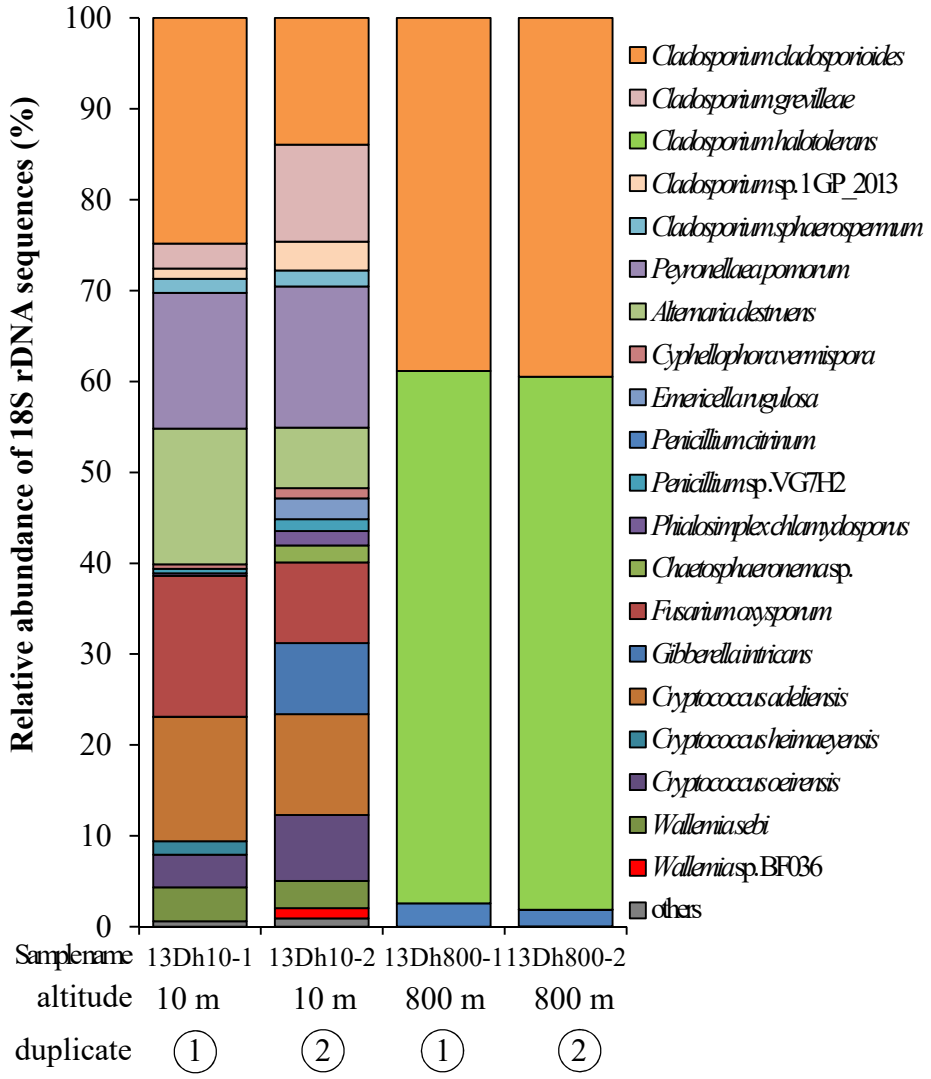


Fig. 4.5 Fungal compositions at species level of the partial sequences in pyrosequencing database (ca 400 bp) obtained from duplicated air sample (1, 2) collected at altitude of 10 m and 800 m over Dunhuang city on September 10, 2013

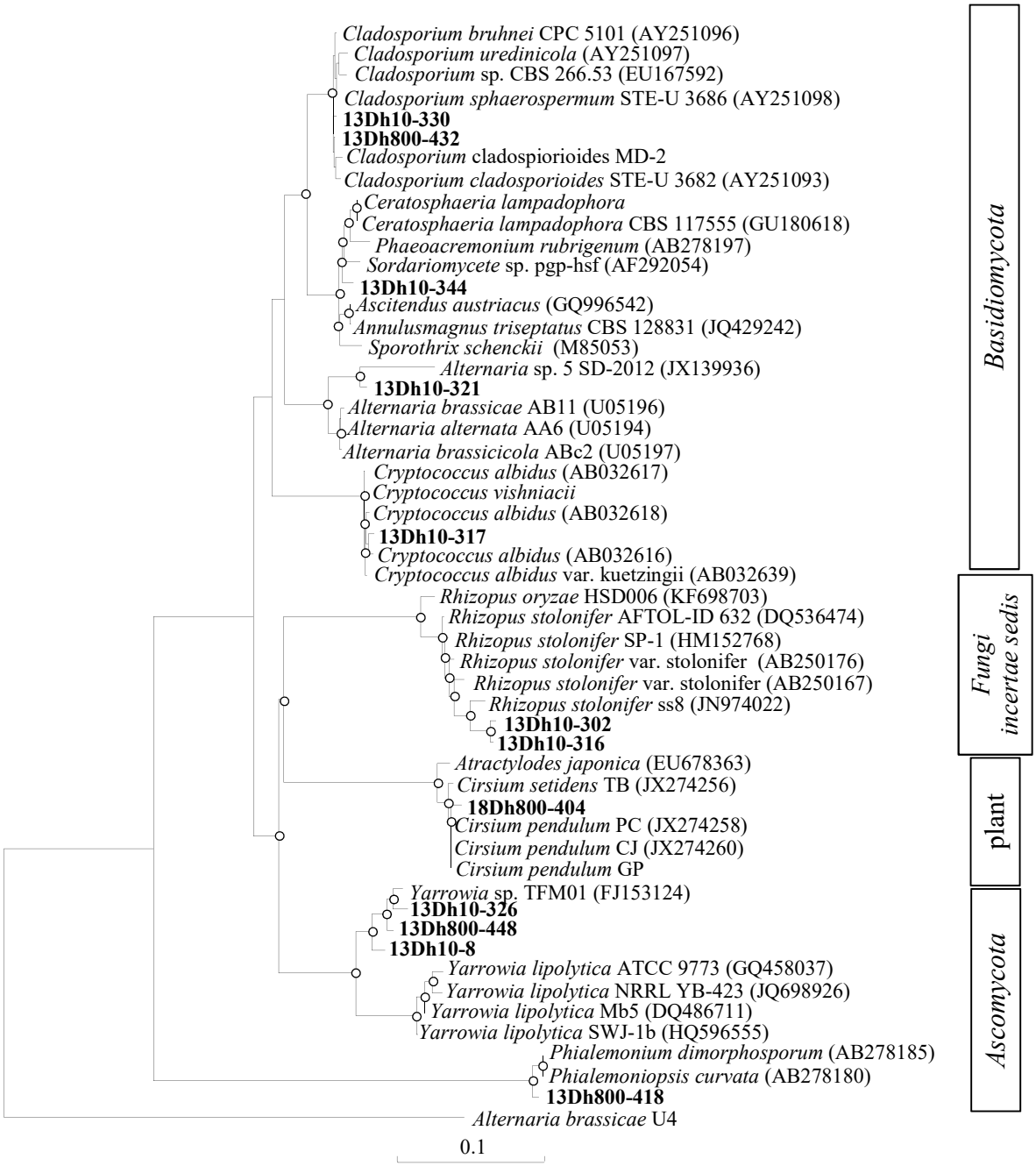


Fig. 4.6 Phylogenetic tree including the partial sequences of 18S rDNA amplicons obtained from the clone libraries (10 m; 13Dh10 and 800 m; 13Dh800). The sample information and the accession number of each reference sequence are given in parentheses. Open circles at branch points show that bootstrap values obtained by neighbor-joining analysis exceeded 50% (after 1000 resamplings)

Chapter 5:

CONCLUSION

In this study, airborne bacteria in Kosa downwind area (Kanazawa city; Japan) are demonstrated to vary the species compositions during the dust events (Chapter 2). In the initial dust phases, the dust mainly included marine cyanobacteria and seawater components. Then, the bacterial communities during the middle of the Kosa event were dominated by the members of the *Firmicutes*, such as *B. subtilis* and *B. pumilus*, which are expected to have been carried by the Kosa event. At the end phase of the Kosa event, the air mass over the north areas of the Sea of Japan and around Japan predominantly included the *Pelagibacter* spp. (SAR clade) and *Sphingomonas* spp. that are possibly transported the marine area, and *B. megaterium* that are thought to originate from the local bacterial population became predominant. Moreover, the bacterial biomasses were also

changed by immigrations of bacterial populations associated with dust mineral particles and marine bacterial populations during Kosa events.

The bacterial populations that increased over Kosa downwind area were detected from dune sand in the Kosa source region, such as Taklamakan desert (Chapter 3). The airborne bacterial population at a dust source region also changed after dust events was beginning. *Bacillus* species, which that detected in Kosa downwind region, increased in correspondence to dust events. The populations of *Bacillus* species were also dominated in the dune sand samples of desert areas. Although some members of the phyla *Actinobacteria* were also dominated in the sand samples, they decreased during the dust event or disappeared in atmosphere, suggesting the elimination of bacterial population by atmospheric stressors.

Furthermore, for understanding of vertical mixture processes of airborne bacterial communities at high altitudes in Kosa source, microbial community structures in the bioaerosol samples collected at 800 m and 10 m were analyzed using the DNA sequence information of bacterial 16S rRNA genes and fungal ITS regions (Chapter 4). Bacterial compositions in bioaerosol sample were similar species between the both altitudes and dominated by the members of phyla *Firmicutes* (*Bacillus* species) and *Gamma-proteobacteria*. These results also supported the suggestion that Bacillus population possibly transported from China desert are to the downwind area for long-range distance. The dominant population of fungi (*Cladosporium* species) in the sample of 800 m were commonly detected from the samples of 10 m, and those of 10 m were composed of the higher diverse members of two phyla *Ascomycota* and *Basidiomycota*. These results suggested

that fungal populations transported from ground surfaces air to atmosphere and that some population such as *Cladosporium* species and *Penicillium* species remain at high altitude of 800 m. In the samples of 800 m, the dominated levels of fungal populations were higher than those of bacterial population, suggesting that the most fungal populations would be hardly transported to higher altitudes.

The some bacterial population originated from dune sand would be transported to atmosphere over Kosa source region, and potentially immigrate to Kosa downwind areas maintaining their viabilities. The bacterial communities around downwind areas such as Japan exhibit significant changes in species compositions depending on Kosa event occurrences and the origin of air masses. The microbial sequences, which were commonly detected from the both of Kosa source and arrival regions, were related to several species that were found to be associated with plant and animal growth, human health, geochemical processes, and organic-matter cycles. In the future, the impact of airborne bacterial populations on human societies and bio-ecosystems in Kosa downwind environments should be investigated using physiological experiments targeting bacterial cultures and molecular biological analysis using multiple functional genes.

REFERENCES

Abdel HA (2003) Air-borne particulate matter and its viable fraction during severe weather condition in Cairo, Egypt. B Serisi FenBilimeri 4:1–8

Amato P, Parazols M, Sancelme M, Mailhot G, Laj P, Delort AM. (2007) An important oceanic source of micro-organisms for cloud water at the Puy de Dôme (France). Atmos Environ 41:8253–6823

An S, Couteau C, Luo F, Neveu J, DuBow MS (2013) Bacterial diversity of surface sand samples from the Gobi and Taklamaken deserts. Microb Ecol 66:850-860 doi:10.1007/s00248-013-0276-2

Baraniecki CA, Aislabie J, Foght JM (2002) Characterization of *Sphingomonas* sp. Ant 17, anaromatic hydrocarbon-degrading bacterium isolated from Antarctic soil. Microb Ecol;43:44–54

Boreson J, Dillner AM, Peccia J (2004) Correlating bioaerosol load with PM2.5 andPM10cf concentrations: A comparison between natural desert and urban-fringe aerosols.Atmos Environ 38:6029–6041

Berg G, Roskot N, Smalla K (1999) Genotypic and phenotypic relationships between clinical and environmental isolates of *Stenotrophomonas maltophilia*. J. Clin. Microbiol 37 11:3594-3600

Bowers RM, Lauber CL, Wiedinmye C, Hamady M, Hallar AG, Fall R, Knight R, Fiere, N (2009) Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei. Appl. Environ. Microbiol. 75:5121–5130

Brodie EL, DeSantis TZ, Parker JPM, Zubietta IX, Piceno YM, Andersen GL (2007) Urban aerosols harbor diverse and dynamic bacterial populations. Proc. Natl. Acade. Sci. USA 104:299–304

Brown JKM, Hovmøller MS (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297:537–41

Bryant MP (1959) Bacterial species of the rumen. Bacteriol Rev 23:125-153

Chakraborty P, Gupta BS, Chowdhury I, Majundar MR, Chanda S (2001) Differences in concentrations of allergenic pollens ans spore at different

heights on an agricultural farm in West Bengal India. Annals of Agricultural and Environmental Medicine 8:123-130

Chanal A, Chapon V, Benzerara K, Barakat M, Christen R, Achouak W, Barras F, Heulin T (2006) The desert of Tataouine: an extreme environment that hosts a wide diversity of microorganisms and radio tolerant bacteria. Environ Microbiol 8:514-525

Chao HJ, Chan CC, Rao CY, Lee CT, Chuang YC, Chiu YH, Hsu HH, Wu YH (2012) The effects of transported Asian dust on the composition and concentration of ambient fungi in Taiwan. Int J Biometeorol 56:211-219

Choi DH, Noh JH (2009) Phylogenetic diversity of *Synechococcus* strains isolated from the East China Sea and the East Sea. FEMS Microbiol Ecol 69:439-448

DeLeon-Rodriguez N, Lathem TL, Rodriguez-R LM, Barazesh JM, Anderson BE, Beyersdorf AJ, Ziemba LD, Bergin M, Nenes A, Konstantinidis KT (2013) Microbiome of the upper troposphere: Species composition and prevalence, effects of tropical storms, and atmospheric implications. Proc. Natl. Acade. Sci. USA 110:2575–2580

Despres VR, Nowoisky JF, Klose M, Conrad, Andreae MO, Pösch U (2007) Characterization of primary biogenic aerosol particles inurban, rural, and high-alpine air by DNA sequence and restriction fragment analysis ofribosomal RNA genes. Biogeosciences 4:1127–1141

Dieser M, Greenwood M, Foreman CM (2010) Carotenoid pigmentation in antarctic heterotrophic bacteria as a strategy to withstand environmental stresses. Arct Antarct AlpRes 42:396–405

Delort AM, Vaitilingom M, Amato P, et al. (2010) A short overview of the microbial population in clouds: potential roles in atmospheric chemistry and nucleation processes. Atmospheric Research 98:249-260

Duce RA, Unni CK, Ray BJ, Prospero JM, Merrill JT (1980) Long-range atmospheric transport of soil dust from Asia to the tropical North Pacific: temporal variability. Science 209:1522–1524

Eguchi M, Nishikawa T, McDonald K, Cavicchioli R, Gottschal JC, Kjelleberg S (1996) Response to stress and nutrient availability by the marine

ultramicrobacterium *Sphingomonas* sp. strain RB2256. Appl Environ Microbiol 62:1287–1294

Fierer N, Liu Z, Hernández MR, Knight R, Henn M, Hernandez MT (2008) Short-term temporal variability in airborne bacterial and fungal populations. Appl Environ Microbiol 74:200–207

Fuller NJ, Marie D, Partensky F, Vaulot D, Post AF, Scanlan DJ (2003) Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine *Synechococcus* clade throughout a stratified water column in the Red Sea. Appl Environ Microbiol 69:2430–2443

Fürnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A (2008) Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. ISME J 2 5:561–570

Gill SR, Fouts DE, Archer GL, Mongodin EF, DeBoy RT, Ravel J, Paulsen IT, Kolonay, JF, Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J, Tran K, Kang K, Hance IR, Nelson KE, Fraser CM (2005) Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J. Bacterio 187:2426–2438

Giovannoni SJ, Stingl U (2005) Molecular diversity and ecology of microbial plankton. Nature 437:343–348

Gloeckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, et al. (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of *Actinobacteria*. Appl Environ Microbiol 66:5053–5065

Good IJ (1953) The population frequencies of species and the estimation of population parameters. Biometrika 40:237–264

Griffin DW, Kellogg CA, Garrison VH, Lisle JT, Borden TC, Shinn EA (2003) Atmospheric microbiology in the northern Caribbean during African dust events. Aerobiologia 19:143–157

Hara K, Zhang D (2012) Bacterial abundance and viability in long-range transported dust. Atmos Environ 47:20–25

Ho HM, Rao CY, Hsu HH, Chiu YS, Liu CM, Chao HJ (2005) Characteristics and determinants of ambient fungal spores in Hualien, Taiwan. Atmos Environ 39:5839–5850

Hold GL, Pryde SE, Russell VJ, Furrie E, Flint HJ (2002) Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. FEMS Microbiol Ecol 39:33-39

Hou CT, Labeda DP, Rooney AP (2005) Evaluation of microbial strains for linoleic acid hydroxylation and reclassification of strain ALA2. Antonie Van Leeuwenhoek 88:167–171

Hu FP, Young JM, Triggs CM, Park DC, Saul DJ (2001) Relationships within the *Proteobacteria* of plant pathogenic *Acidovorax* species and subspecies, *Burkholderia* species, and *Herbaspirillum rubrisubalbicans* by sequence analysis of 16S rDNA, numerical analysis and determinative tests. Antonie van Leeuwenhoek 80:201–213

Hua NP, Kobayashi F, Iwasaka Y, Shi GY, Naganuma T (2007) Detailed identification of desert-originated bacteria carried by Asian dust storms to Japan. Aerobiologia 23:291–298

Ichinose T, Nishikawa M, Takano H, Sera N, Sadakane K, Mori I, et al. (2005) Pulmonary toxicity induced by intratracheal instillation of Asian yellow dust (Kosa) in mice. Regul Toxicol Pharmacol 20:48–56

Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K (2003) Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist Update 6:41-52

Iwasaka Y, Minoura H, Nagaya K (1983) The transport and special scale of Asian dust-storm clouds: a case study of the dust-storm event of April 1979 Tellus 35B:189–196

Iwasaka Y (2003) Importance of dust particles in the free troposphere over the Taklamakan Desert: Electron microscopic experiments of particles collected with a balloonborne particle impactor at Dunhuang, China. Journal of Geophysical Research

Iwasaka Y, Shi GY, Yamada M, Matsuki A, Trochkine D, Kim YS, Zhang DD, Nagatani T, Shibata T, Nagatani M, Nakata H, Shen Z, Hong CS (2008) Mass transport of background Asian dust revealed by balloon-borne measurement: dust particles transported during calm periods by westerly from Taklamakan Desert. In: Kim YJ, Platt U (eds) Advanced environmental monitoring 121–135

Iwasaka Y, Shi GY, Yamada M, Kobayashi F, Kakikawa M, Maki T, Chen B, Tobo Y, Hong C (2009) Mixture of Kosa (Asian dust) and bioaerosols detected in the atmosphere over the Kosa particles source regions with balloon-borne measurements: possibility of long-range transport. Air Quality Atmosphere and Health 2:29-38

Jaenicke R (2005) Abundance of cellular material and proteins in the atmosphere. Science 308:373

Joly M, Attar, E, Sancelme M, Deguillaume L, Guilbaud C, Morris CE, Amato P, and Delort AM (2013) Ice nucleation activity of bacteria isolated from cloud water. Atmos. Environ.70:392–400

Jeon EM, Kim HJ, Jung K, Kim JH, Kim MY, Kim YP, et al. (2011) Impact of Asian dust events on airborne bacterial community assessed by molecular analyses. Atmos Environ 45:4313–4321

Janssen PH (2006) Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Appl Environ Microbiol 72:1719-1728

Jones AM, Harrison RM. (2004) The effects of meteorological factors on atmospheric bioaerosol concentrations a review. Sci Total Environ 326:151–180

Kakikawa M, Kobayashi F, Maki T, Yamada M, Higashi T, Chen B, et al. (2009) Dustborne micro-organisms in the atmosphere over Asian dust (KOSA) source region, Dunhuang. Air Qual Atmos Health 1:195–202

Kellogg CA, Griffin DW (2006) Aerobiology and the global transport of desert dust Trends. Ecol Evol 21:638-644

Kellogg CA, Griffin DW, Garrison VH, Peak KK, Royall N, Smith RR, Shinn EA (2004) Characterization of aerosolized bacteria and fungi from desert dust

events in Mali, West Africa. Aerobiologia 20:99–110

Kenzaka T, Sueyoshi A, Baba T, Li P, Tani K, Yamaguchi N, Nasu M (2010) Soil Microbial Community Structure in an Asian Dust Source Region (Loess Plateau). Microbes and Environments 25:53-57

Kim J-S, Crowley DE, Buerkert A (2010) Bacterial communities from soil sediments of a mountain oasis in northern Oman Catena 82:102-111

Latifi A, Ruiz M, Zhang CC (2009) Oxidative stress in cyanobacteria. FEMS Microbiol Rev 33:258–278

Li K, Dong S,Wu Y, Yao M (2010) Comparison of the biological content of air samples collected at ground level and at higher elevation. Aerobiologia 26:233–244

Li L, Lei C, Liu ZG (2010) Investigation of airborne fungi at different altitudes in Shenzhen University. Natural Science2 5:506-514

Liu B, Ichinose T, He M, Kobayashi F, Maki T, Yoshida S, Yoshida Y, Arashidani K, Takano H, Nishikawa M, Sun G, Shibamoto T (2014) Lung inflammation by fungus, *Bjerkandera adusta* isolated from Asian sand dust (ASD) aerosol and enhancement of ovalbumin-induced lung eosinophilia by ASD and the fungus in mice. Allergy, Asthma & Clinical Immunology 10:10

Lopetuso LR, Scaldaferri F, Petito V, Gasbarrini A (2008) Commensal Clostridia: leading players in the maintenance of gut homeostasis. Gut Pathogens 5:23

Maidak BL, Olsen GJ, Larsen N, Overbeek R, McCaughey MJ, Woese C (1997) The RDP (ribosomal database project). Nucleic Acids Res 25:109–110

Maki T, Susuki S, Kobayashi F, Kakikawa M, Yamada M, Higashi T, et al. (2008) Phylogenetic diversity and vertical distribution of a halobacterial community in the atmosphere of an Asian dust (KOSA) source region, Dunhuang City. Air Qual Atmos Health 1:81–89

Maki T, Susuki S, Kobayashi F, Kakikawa M, Tobo Y, Yamada M, et al. (2010) Phylogenetic analysis of atmospheric halotolerant bacterial communities at high altitude in an Asian dust (KOSA) arrival region, Suzu City. Sci Total Environ 408:4556–4562

Maki T, Aoki S, Susuki S, Kobayashi F, Kakikawa M, Hasegawa H, et al. (2011)

Characterization of halotolerant and oligotrophic bacterial communities in Asian desert dust (KOSA) bioaerosol accumulated in layers of snow on Mount Tateyama, Central Japan. Aerobiologia 27:277–290

Maki T, Kakikawa M, Kobayashi F, Yamada M, Matsuki A, Hasegawa H, et al. (2013) Assessment of composition and origin of airborne bacteria in the free troposphere over Japan. Atmos Environ 74:73–82

Maki T, Kobayashi F, Yamada M, Hasegawa H, Iwasaka Y (2012) NaCl-amendment assay targeting airborne bacteria in tropospheric bioaerosols transported by westerly wind over Noto Peninsula. Aerobiologia 29:341-354

Maki T, Puspitasari F, Hara K, Yamada M, Kobayashi F, Hasegawa H, Iwasaka Y (2014) Variation in the structure of airborne bacteria communities in a downwind area during Asian dust (Kosa) event. Science of the total environment 488-489:75-84

Miteva VI, Sheridan PP, Brenchley JE (2004) Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. Appl Environ Microbiol 70:202–213

Mohler PJ, Le SS, Denjoy I, Lowe JS, Guicheney P, Caron L, Driskell IM, Schott JJ, Norris K, Leenhardt A, Kim RB, Escande D, Roden DM (2007) Defining the cellular phenotype of "ankyrin-B syndrome" variants: human ANK2 variants associated with clinical phenotypes display a spectrum of activities in cardiomyocyte. Epub 30;115(4):432-412

Morris RM, Rappé MS, Connon SA, Vergin KL, Siebold WA, Carlson CA, et al. (2002) SAR11clade dominates ocean surface bacterioplankton communities. Nature 420:806–810

Mostajir B, Dolan JR, Rassoulzadegan F (1995) A simple method for the quantification of a class of labile marine pico- and nano-sized detritus: DAPI yellow particles (DYP). Aquat Microb Ecol 9:259–266

Nagano Y, Itoh K, Honda K (2012) The induction of Treg cells by gut-indigenous *Clostridium*. *Curr Opin Immunol* 24:392-397

Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial

environments. Microbiol Mol Biol Rev 64:548–572

Nowoisky JF, Pickersgil DA, Despres VR, Poschl U (2009) Hight diversity of fungi in air particulate matter. PNAS 106:12814-12819

Okafor N (2010) Environmental microbiology of aquatic and waste systems. ISBN 978-94-007-1459-5 NewYork Springer

Ostrowski M, Caviccioli R, Blaauw M, Gottschal JC (2001) Specific growth rate plays a critical role in hydrogen peroxide resistance of the marine oligotrophic ultramicrobacterium *Sphingomonas alaskensis* strain RB2256. Appl Environ Microbiol 67:1292–1299

Perelman A, Uzan A, Hacohen D, Schwarz R (2003) Oxidative stress in *Synechococcus* sp. strain PCC 7942: various mechanisms for H_2O_2 detoxification with different physiological roles. J Bacteriol 185:3654–3660

Polymenakou PN, Mandalakis M, Stephanou EG, Tselepides (2008) A. Particle size distribution of airborne microorganisms and pathogens during an intense African dust event in the Eastern Mediterranean. Environ Health Perspect 116:292–296

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic micro flora.Limnol Oceano 25:943–948

Pratt KA, DeMott PJ, French JR, Wang Z, Westphal DL, Heymsfield AJ, et al. (2009) Insitu detection of biological particles in cloud ice-crystals. Nat Geosci 2:398–401

Prospero JM, Blades E, Mathison G, Naidu R (2005) Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. Aerobiologia 21:1–19

Rodó X, Ballester J, Cayan D, Melish ME, Nakamura Y, Uehara R, et al. (2011) Association of Kawasaki disease with tropospheric wind patterns. Sci Rep 2011;1:152

Saitou N and Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sattler B, Puxbaun H, Psenner R (2001) Bacterial growth in supercooled cloud droplets. Geophys. Res. Lett. 28(2):239-242

Senol E (2004) Stenotrophomonas maltophilia: the significance and role as a nosocomial pathogen. J Hosp Infect 57:1-7 doi:10.1016/j.jhin.2004.01.033

Shinn EA, Griffin DW, Seba DB (2003) Atmospheric transport of mold spores in clouds of desert dust. Arch Environ Health 58:498–504

Smith DJ, Jaffe DA, Birmele MN, Griffin DW, Schuerger AC, Hee J, et al. (2012) Free tropospherictransport of microorganisms from Asia to North America. Microb Ecol 64:973–85

Solomon PA, Moyers JL, Fletcher RA (1983) High-volume dichotomous virtual impactorfor the fractionation and collection of particles according to aerodynamic size. Aerosol Sci Tech 2:455–464

Song J, Oh HM, Cho JC (2009) Improved culturability of SAR11 strains in dilution to extinction culturing from the East Sea, West Pacific Ocean. FEMS Microbiol Lett ;295:141–147

Sun J, Zhang M, Liu T (2001) Spatial and temporal characteristics of dust storms in China and its surrounding regions, 1960–1999: Relations to source area and climate. Journal of Geophysical Research

Spain AM, Krumholz LR, Elshahed MS (2009) Abundance, composition, diversity and novelty of soil Proteobacteria. ISME J 3:992-1000

Tripp HJ (2013) The unique metabolism of SAR11 aquatic bacteria. J Microbiol 51:147-153

Uematsu M, Duce RA, Prospero JM, Chen JQ, Merill, JT, McDonald RL (1983) Transport of mineral aerosol from Asia over the North Pacific Ocean. J Geophys Res 88:5343–5352

Uno I (2004) Numerical study of Asian dust transport during the springtime of 2001 simulated with the Chemical Weather Forecasting System (CFORS) model. Journal of Geophysical Research 109 doi:10.1029/2003jd004222

Vaïtilingom M, Attard E, Gaiani N, Sancelme M, Deguillaume L, Flossmann AI, Amato P, Delort AM (2012) Long-term features of cloud microbiology at the puy de Dôme (France). Atmos. Environ. 56:88–100

Wang W, Ma Y, Ma X, Wu F, Ma X, An L, Feng H (2010) Seasonal variations of airborne bacteria in the Mogao Grottoes, Dunhuang, China. International Biodeterioration and Biodegradation 64:309-315

Wang W, Ma Y, Ma X, Wu F, Ma X, An L, Feng H (2012) Diversity and seasonal dynamics of airborne bacteria in the Mogao Grottoes, Dunhuang, China. Aerobiologia, 28:27-3855

Weir-Brush JR, Garrison VH, Smith GW, Shinn EA (2004) The relationship between gorgonian coral (Cnidaria: Gorgonacea) diseases and African dust storms. Aerobiologia 20:119–126

Womack AM, Bohannan BJM, Green JL (2010) Biodiversity and biogeography of the atmosphere. Philos. Trans. R. Soc. B-Biol. Sci. 365:3645–3653

Wu PC, Tsai JC, Li FC, Lung SC, Su HJ (2004) Increased levels of ambient fungal spores in Taiwan are associated with dust events from China. Atmos Environ 38:4879–4886

Yamaguchi N, Ichijo T, Sakotani A, Baba T, Nasu M (2012) Global dispersion of bacterial cells on Asian dust. Sci Res 2:525

Yeo HG, Kim JH (2002) SPM and fungal spores in the ambient air of West Korea during the Asian dust (Yellow sand) period. Atmos Environ 36:5437–5442

Zhang D, Iwasaka Y, Matsuki A, Ueno K, Matsuzaki T (2006) Coarse and accumulation mode particles associated with Asian dust in southwestern Japan. Atmos Environ 40:1205–1215

Zwart G, Crump BC, Agterveld MPKV, Hagen F, Han SK (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol 28:141-155

Acknowledgment

The author would like to express her biggest gratitude to Dr. Maki Teruya (Associate Professor, Institute of Science and Engineering, Kanazawa University, Japan), as her chief supervisor for his kindly help, outstanding guidance, and advice in her study during her doctoral work in Kanazawa University. Furthermore, the author would like to thankful to Prof. Hiroshi Hasegawa (Professor, Institute of Science and Engineering, Kanazawa University, Japan) for his support and guidance.

The author wishes to thank to Fumihisa Kobayashi (College of science and Engenering, Kanazawa University, Japan), Yasunori Kurosaki (Arid land Research Center, Totori University, Japan), Maiko Kakikawa and Atsushi Matsuki (Institute of Nature and Environmental Technology, Kanazawa University, Japan), Chen Bin and Guangyu Shi (Institute of Atmospheric Physics, Chinese Academy of Science, China), Yasunobu Iwasaka (Community Research Service Group, University of Shiga Prefecture, Japan), Rie Fukushima (Fukumitsuya, Japan) for the support and valuable suggestions.

She also express her thankful to her sponsorship, Indonesian Scholarship DIKTI under the KU-DIKTI Program Student 2012. During her study, the author also be supported and helped by International Affair and Graduate School of Natural Science and Technology office. Special thanks also been expressed to the entire laboratory member, who support her during the entire period of research work.

Finally, she wishes to thank sincerely to her lovely parents, brothers, others relatives and friends for outstanding loving and support.