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Abstract

Airborne microorganisms (bioaerosol) from the China desert region, which are released into the atmosphere, disperse by the Asian dust event and affect ecosystems, human life, and atmospheric processes in downwind areas. However, the dynamics of airborne bacteria over the China desert regions have rarely been investigated. In this study, we analyzed bacterial communities in aerosols of the Asian dust source region (Taklimakan Desert) and compared them with the bacterial communities in sand dunes, in order to evaluate the differences between the mixtures from the sand and from the atmosphere. Air samples were collected at 10 m above the ground level from Dunhuang city, during a dust event. The cell densities of airborne bacteria during a dust event were ten times more than that in non-dust periods. The 16S rDNA clone libraries from four air samples mainly belonged to two phyla, *Firmicutes* and *Proteobacteria*. During a dust event, the proportion of *Proteobacteria* clones decreased, whereas that of *Firmicutes* clones increased. Sand samples were collected from the sand dunes in four sampling sites of the Taklimakan Desert. The bacterial communities in sand samples comprised members of *Firmicutes* and *Actinobacteria*. The clones of *Firmicutes* in both air and sand samples included *Bacillus* species, constituting more than 10% of total clones. Airborne bacterial communities are likely carried by the dust events from sand dunes. *Propionibacterium* species from the class *Actinobacteria* that were dominant in sand samples were not detected in the air samples, suggesting that atmospheric stressors eliminate some bacterial species. Presumably, airborne bacterial communities in the Asian dust source region are composed of local environmental bacteria, and their dynamics depend on the occurrence of a dust event.

Keywords: Asian dust, bacteria, *Bacillus*, sand dune

Introduction

Soil particles that are derived from the Chinese desert area are frequently transported over the East China Sea or the Yellow Sea to Japan during the spring season (Iwasaka et al. 1983). These Asian dust clouds, which are common atmospheric phenomena in Japan, are referred to as "Kosa," which 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; Kim et al. 2010), causing damage to human health (Ichinose et al. 2005). Furthermore, these transoceanic and transcontinental dust events inject a large pulse of microorganisms and pollen (bioaerosol) into the atmosphere (Iwasaka et al. 2008; Kakikawa et al. 2008). The dispersal of bioaerosol by the Asian dust event could have a role in transporting pathogens or expanding the biogeographical range of some organisms by facilitating long-distance dispersal events (Kellogg and Griffin 2006; Kobayashi et al. 2015; Chung and Kim 2008). Results from *in vivo* assays in mice have also suggested that the Asian dust events transport microbial matter and allergens that potentially affect the health of downwind populations and ecosystems (Liu et al. 2014).

The major source areas of the Asian dust event are the Taklimakan Desert, Gobi Desert, and Loess Plateau (Sun et al. 2001; Duce et al. 1980; Iwasaka et al. 1983). Dust storms from the China desert area likely inject dust particles, which are transported at high altitudes for long distances, into the upper atmosphere (Iwasaka et al. 2003; Uno et al. 2004; Huang et al. 2010). Some studies reported that bacterial communities in the

Asian dust 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 Tarim basin (Taklimakan Desert), is an optimal site to study the dynamics and composition of the Asian dust with microorganisms released from the Taklimakan 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 Taklimakan 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 the atmosphere of the Asian dust source region (Taklimakan Desert) were investigated to identify and assess 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 Taklimakan Desert to establish the source of bacterial populations in the atmosphere over dust regions.

Materials and Methods

Sampling of aerosol 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 Taklimakan 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 Taklimakan 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. 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. In the Ming Sha Mountain, located 15 km from Dunhuang city, it was likely that there was some influence due to human activity in Dunhuang city. Peacock Rock and Sphinx Rock were in the Geological National Park (Taklimakan 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 ℃.

Characteristics and trajectories 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 air sampling area for this study (40.21°N, 94.68°E), from a site 10 m above the ground level to estimate the trajectories of air masses in the boundary layer.

Microscopic analysis of particle abundance

To determine the particle abundance 0.25 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.

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 Polymerase Chain Reaction (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).

Bacterial isolation from aerosol 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 K_2 PO_4, 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).

Accession numbers

The DDBJ accession numbers for the 16S rDNA sequences determined in this study are from AB924227 to AB924368 (Table 1) and LC055609 to LC055615 (Table 2).

Results and Discussion

Transport trajectory and environmental factors

Analysis of air-mass backward trajectory across three days (September 9 and 11, 2012) indicated that the air mass source was carried from the Taklimakan Desert to the sampling sites at the Dunhuang city Meteorological Department in (Fig. 2). During air sampling period, temperature fluctuated between 7°C and 29°C, and relative humidity ranged from 19% to 73% (Fig. 3). Air samples were collected on the roof of the Dunhuang City Meteorological Department, 10 m above the ground and on two different days (September 10 and 11, 2012). Concentration of the particles showed some variation during sampling times, and the concentrations of particle slightly increased in the second day of sampling when the dust event occurred (Fig. 3). Bacterial abundance was determined using fluorescence microscopy 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, while bright-blue fluorescence particles were bacteria with smaller diameters <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. 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 Asian dust 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. 4).

Bacterial composition in air samples

After 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 *Escherichia coli*, and 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 1). The bacterial communities in air-sample clones consisted of similar species among the four samples, and the ratio of clones indicated bacterial dynamics during the sampling period. Most of the phylotypes from the air samples belonged to the phyla *Firmicutes*, *Proteobacteria*, and *Cyanobacteria* (Fig. 5).

Bacillus subtilis in *Firmicutes* constituted almost 30% of the clones in DhA3 and DhA4, and this ratio was greater than that during non-dust periods. The isolates of *B. subtilis* were also collected from the air samples and identified using the clone sequences (Fig. 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 their 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 Asian dust events (Jeon et al. 2011). Some halotolerant bacteria isolated from sand dunes in the Gobi Desert belonging to the genus *Bacillus*, were isolated in Higashi-Hiroshima, Japan, indicating the possibility of their long-range transport (Hua et al. 2007). Sugimoto et al. (2012) demonstrated vertical mixture of bioaerosol by dust event in downwind area, and the bacterial that maintained in the atmosphere have high possibility to be transported and deposit to downwind area. In the air samples DhA1 and DhA2 obtained during non-dust periods, alpha proteobacterial populations from *Proteobacteria*, and unidentified *Cyanobacteria* constituted more than 40% of the clones and this ratio halved in non-dust periods. *Proteobacteria* and *Cyanobacteria* have been considered as plant-associated bacteria (Hu et al 2001; Fürnkranz et al. 2008), and are the most common phyla in highly saline marine (abundance between 50% and 80%) (Desriac et al. 2013) and 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 *Firmicutes* were related with *Clostridium* species and were detected only in the air samples. Commensal *Clostridium* are gram-positive, rod-shaped bacteria in the phylum *Firmicutes* and constitute 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-habitated areas of Dunhuang city.

In addition to *Firmicutes*, clones of the phylum *Proteobacteria* were commonly detected in air samples predominated by 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 the desert area would be the 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 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.

Bacterial composition of 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 1). The bacterial

species composition was not significantly different across the four sand samples obtained from the Taklimakan Desert (Magao Cave, Ming Sha Mountain, Peacock Rock, and Sphinx Rock). The majority of the phylotypes from sand samples comprised members of the phyla *Actinobacteria*, *Firmicutes,* and *Proteobacteria*. The most dominant bacterial species in sand samples were *Propionibacterium* sp. of *Actinobacteria*, followed by *B. subtilis* of *Firmicutes,* and *Stenoxybacter* sp. of *Proteobacteria* (found specifically in deserts). Previous studies have reported that the most common phyla present in the soil samples collected from Taklimakan and Gobi deserts were *Firmicutes*, *Proteobacteria*, *and Bacteroidetes*, and *Actinobacteria* (An et al. 2013). Similar phyla were observed in soil samples from other continental deserts such as Tataoune (Chanal et al. 2006). In the Loess plateau, where high amounts of dust particles are accumulated and eventually settle into the soil, 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, while the members of *Bacteroidetes*, *Firmicutes*, and *Planctomycetes* were generally less abundant (Janssen 2006; Kim et al. 2010). Some bacterial species that were previously reported in samples from the desert areas were not observed in the four sand samples in this study. This could be attributed to variations in the environmental conditions in desert areas and the different methods used to estimate bacterial diversity.

Comparison of dominant bacterial species in air and sand samples

The majority of the phylotypes from the sand and air samples belonged to the phyla *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Cyanobacteria*, and *Bacteroidetes*. In both sand and air samples, the clones of *B. subtilis* (phylum *Firmicutes*) dominated, constituting more than 10% of the total clones. *B. subtilis* was possibly transported from the sand dunes to the atmosphere. In total, 8 isolates, which were obtained from the sand and air samples using the 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 2, Fig. 6). The most dominant clones recovered from air samples were those of *B. subtilis*, followed by *Proteobacteria* members, and *Staphylococcus* sp. (Table 1). Bacterial species related to *B. pumilus* and *Staphylococcus* spp. were detected in air samples collected at both 10 and 800 m above the ground in the Asian dust source region of Dunhuang city (Maki et al. 2008). Furthermore, the most dominant clones recovered from sand samples were those of *Propionibacterium* sp., followed by *B. subtilis* and *Stenoxybacter* sp. (Table 1). *Propionibacterium* sp. constituted almost 40% of the clones in sand samples, while they disappeared from air samples. *Propionibacterium* species is a gram-positive, non-spore forming anaerobic bacterium, which is known to be a skin commensal (Levy et al. 2008). It is suggested that this bacterial population cannot survive the atmospheric stresses in desert areas and have been eliminated during the transport process. Some elimination possibly occurs in the early stage of atmospheric transportation.

Conclusion

This study has characterized the airborne bacterial dynamics in the atmosphere at a dust source region (Taklimakan Desert) and compared the airborne bacterial communities with the bacterial communities from the sand of desert areas. Air samples mainly comprised *Firmicutes* and *Proteobacteria*, and the proportion of *Proteobacteria* decreased during a dust event whereas that of *Firmicutes* clones increased correspondingly. *B. subtilis* (*Firmicutes*) was predominant in both sand and air samples. Some bacterial species such as *Actinobacteria* species that were predominant in the sand samples were not detected in the air samples at all, suggesting the elimination of bacterial population by atmospheric stressors. Thus, in future, it is necessary to clarify the details of this elimination processes on the basis of combined spatial and temporal measurements of solar UV intensity, relative humidity, wind speed, temperature, and microorganism divergence in the ground surface atmosphere. Further work characterizing the bacterial communities collected at high altitudes should be analyzed to clearly identify the bacterial communities that have a high possibility to be transported long distances by a dust event.

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Figure Legends

Fig. 1 Sampling site (Dunhuang city) and Kosa (Asian desert dust) source regions (Taklimakan Desert).

Fig. 2 Backward trajectories of air masses containing aerosols in Dunhuang city over three days (September 9 and 11, 2012).

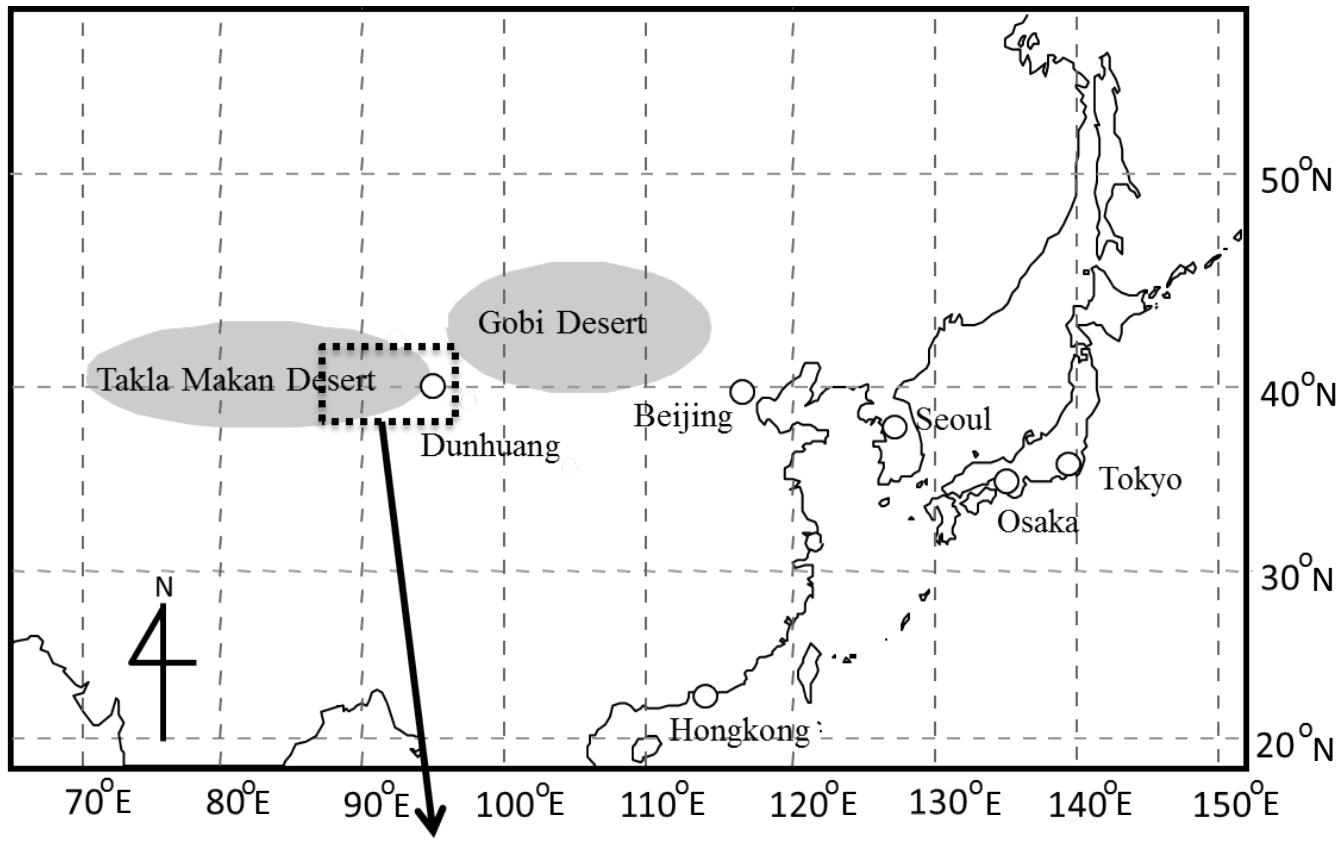
Fig. 3 Concentrations of aerosol particle, temperature, and relative humidity, in the atmosphere 10 m above the ground, in Dunhuang city (10-12 September, 2012).

Fig. 4 Particle concentrations in the atmosphere 10 m above the ground at Dunhuang city Meteorological Department campus (10-12 September 2012).

Fig. 5 Composition of bacterial species in sand and air samples based on clone libraries of bacterial 16S rDNA.

Fig. 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 *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. 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).



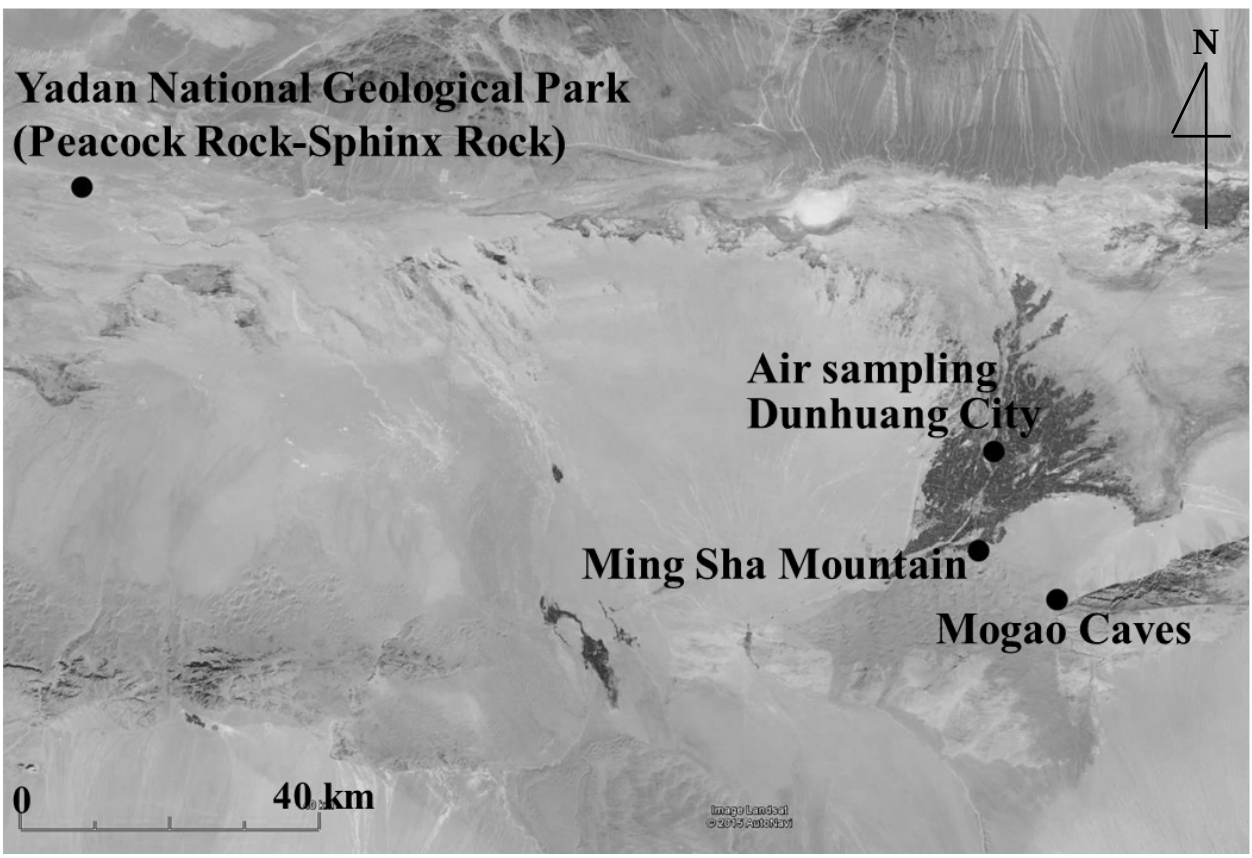
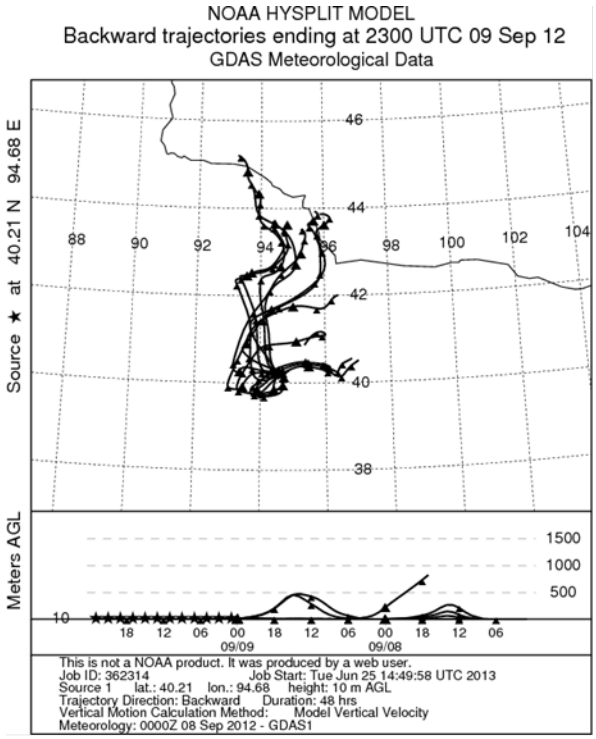
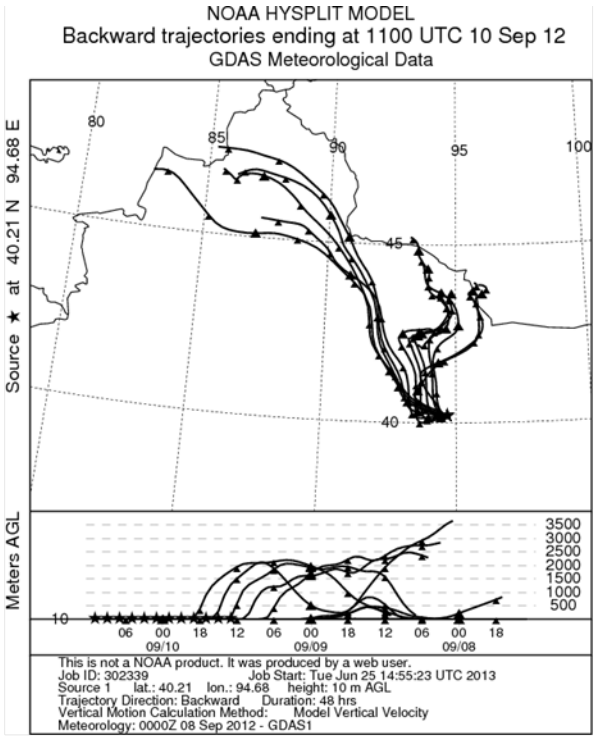
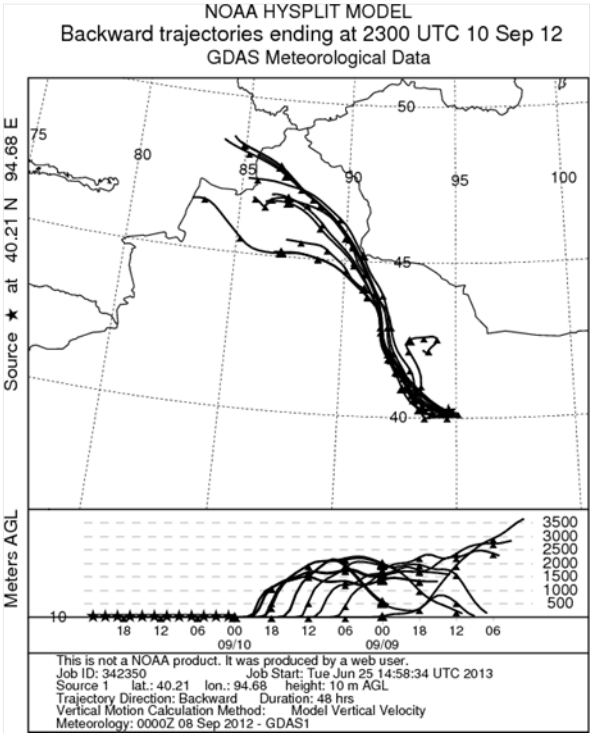
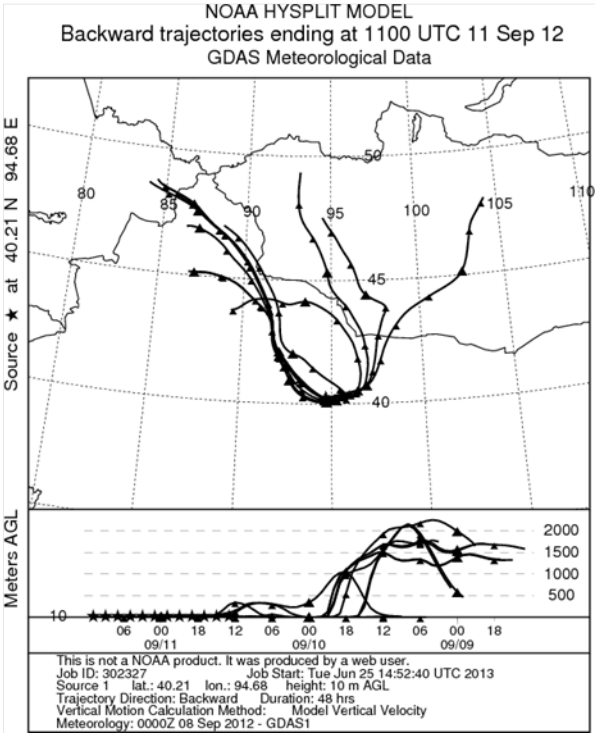


Fig. 1 Findya. P. et al.









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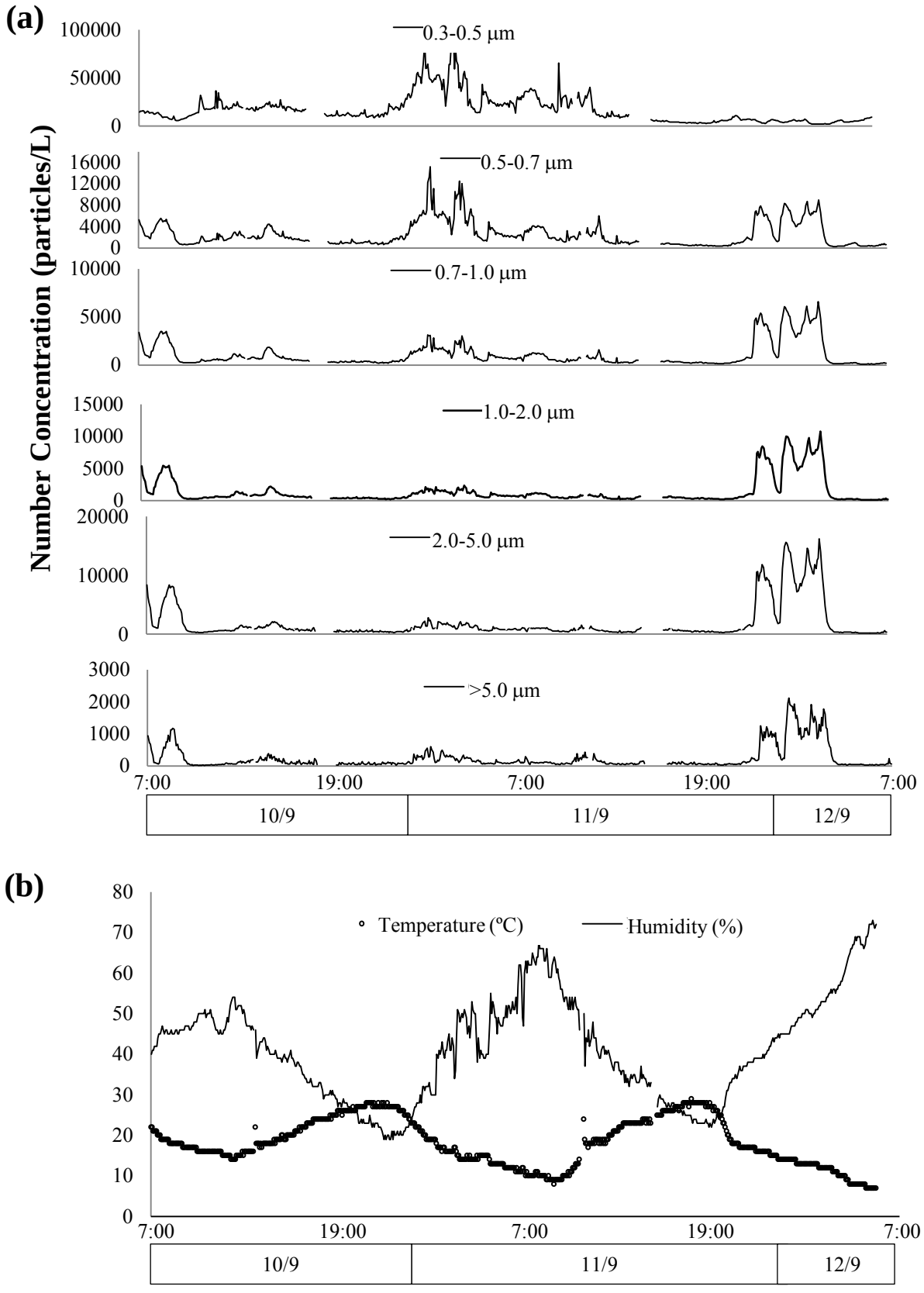
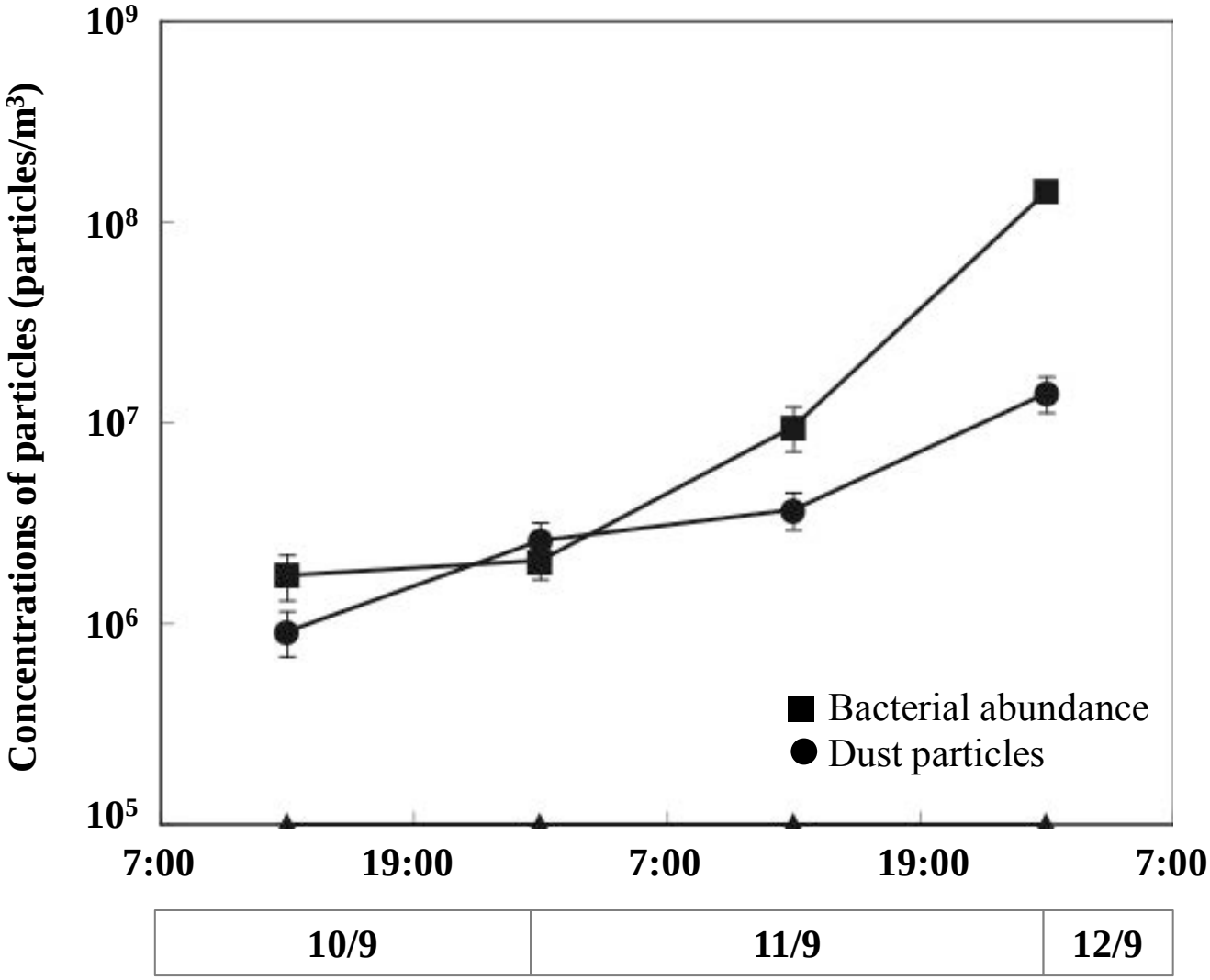


Fig. 3 Findya. P. et al.



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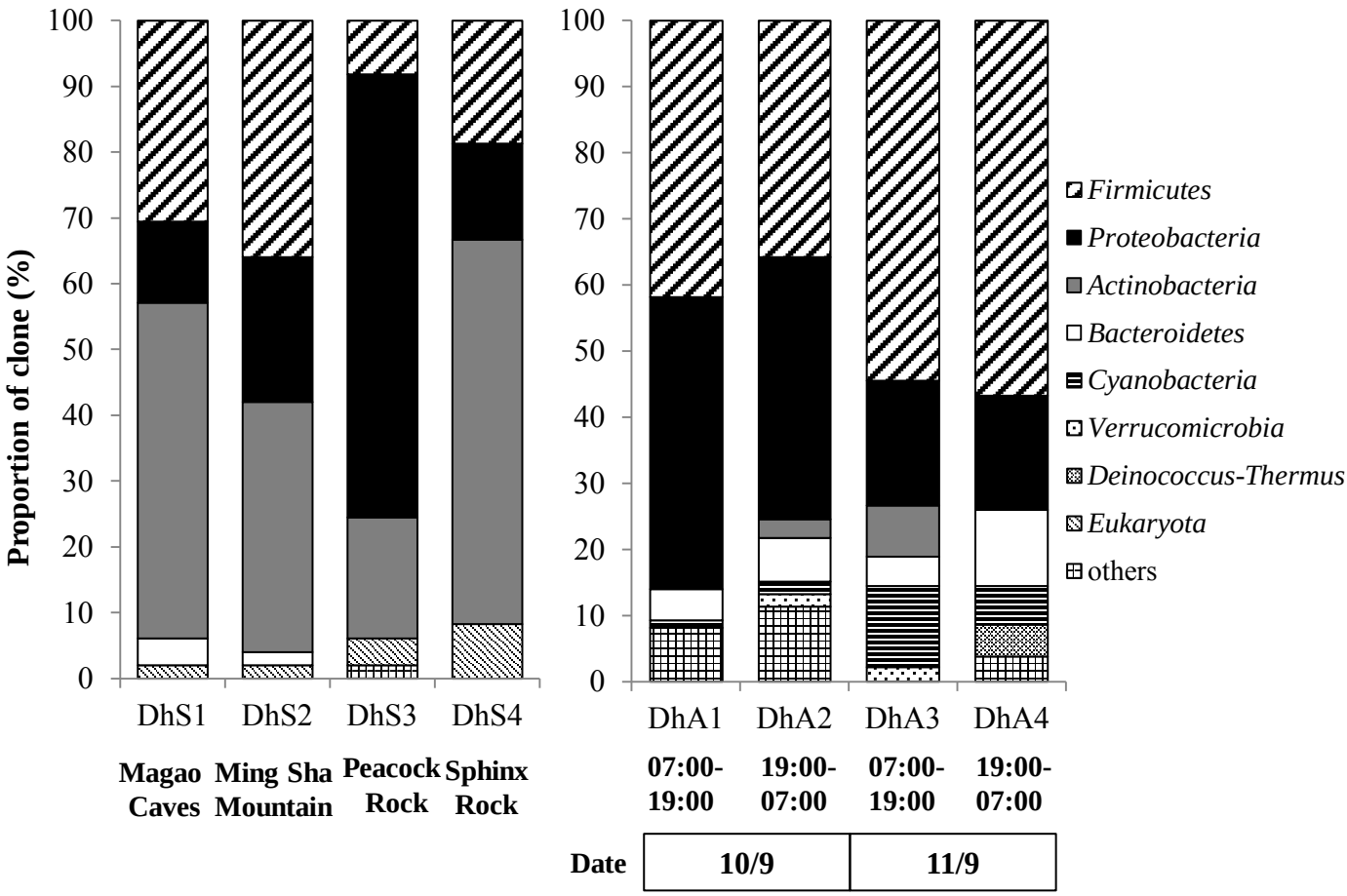

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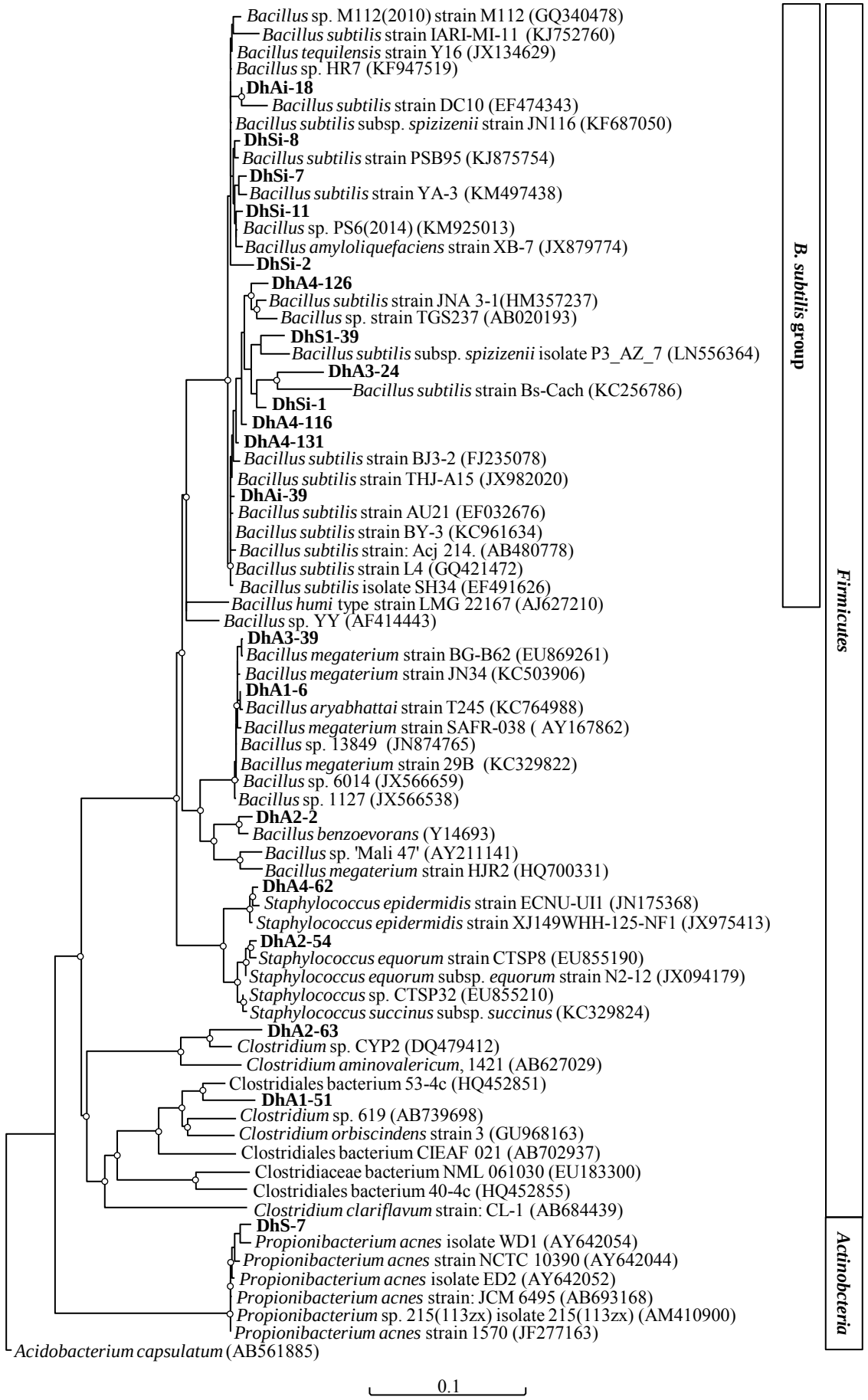

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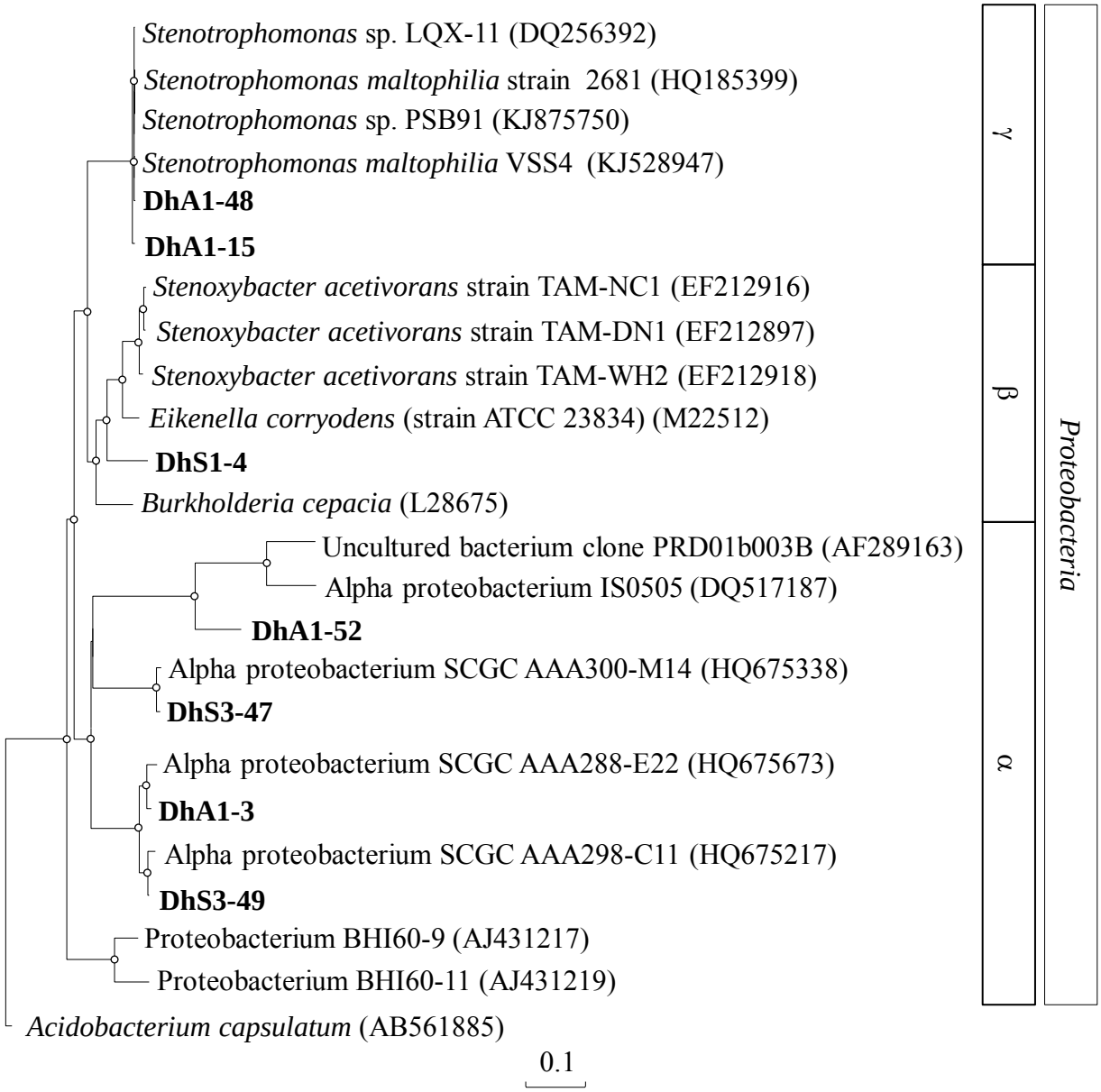

Fig. 7 Findya. P. et al.

Table 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. WS1 (HE60692)	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
	DhS1-25	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 (FQ312002)	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 sequence and the closest relative in databases

Table 2. Phylogenetic affiliation of sequences of bacterial isolates obtained from sand and air samples

Isolate names	Sampling site	Closest relative	Category	Similarity (%)*)	Length (bp)	GenBank accession no.
DhSi-1	Taklimakan Desert	*Bacillus subtilis* (LN556364)	*Firmicutes*	99	675	LC055609
DhSi-2	Taklimakan 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