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ORIGINAL ARTICLE

Modification of atmospheric sand-associated bacterial communities during Asian sandstorms in China and South Korea

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The transport of desert soil into the atmosphere during desert sandstorms can affect the Earth's climate and environmental health. Asian desert sandstorms occur almost every year during the Spring, as the atmosphere in the Northern hemisphere warms. It is conceivable that these Asian desert sandstorms may transport microbes from deserts, such as the Gobi and Taklamaken deserts, over long distances in China, east Asia and the Pacific. In this study, we examined local atmospheric sand particle-associated bacterial populations collected in the absence (sterile sand exposed for 24 h to the air in the absence of a sandstorm) and presence of sandstorms in five Asian cities. We used pyrosequencing of PCR-amplified 16S rDNA genes from sand-extracted total DNA to overcome cultivation limitations of bacterial enumeration. We found that >90% of the control and sandstorm sequences could be classified as representing bacteria belonging to four phyla: *Proteobacteria*, *Bacteriodetes*, *Actinobacteria* and *Firmicutes*. The sand-associated bacterial populations in sandstorm samples were distinct from sand-associated bacteria in the absence of a sandstorm. Members of the phylum *Proteobacteria* were found to significantly increase in sandstorm samples ($P=0.01$). Principal component analyses showed that the sand-associated bacterial populations were best clustered by sampling year, rather than location. DNA sequences representing bacteria belonging to several genera (including putative human pathogens) were observed to increase in sand-associated samples from sandstorms, whereas others were found to decrease, when comparing sand-associated bacterial populations versus those in control samples, suggesting human/environmental implications of sandstorm events.
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INTRODUCTION

Asian sandstorms can spread ~4–8 × 10^{11} kg of sand to other parts of the world every year (Bishop *et al.*, 2002). It is likely that the arrival of desert bacteria on the sand may modify the local atmospheric bacterial population engendering possible human and environmental health effects (Cao *et al.*, 2014). Studies of Asian sandstorm particle-associated bacterial populations may allow the identification of potential microbiological risks to human and environmental health that the sandstorms may bring. The largest sources on Earth for desert sandstorms are the Sahara desert in North Africa and the Gobi plus Taklamaken deserts in Asia (Griffin, 2007). With high-energy winds, the desert sand can be transported over long distances, which makes these sandstorms a global phenomenon. African sandstorms from the Sahara desert and Sahel region can affect the air quality of the Middle East, Europe, Asia, the Caribbean and the Americas (Gorbushina *et al.*, 2007). Asian sandstorms from the Gobi, Taklamaken and Badain Jaran deserts can cross eastern Asia, the Pacific Ocean and arrive in North America (Bishop *et al.*, 2002). Desert dust is an important part of the atmospheric aerosol in some regions, and dust clouds transported over long distances can make substantial contributions to the aerosol content of the atmosphere in distant regions. For example, ~50% of the particles in Florida's atmosphere, each summer, are postulated to be African in origin (Prospero, 1999; Prospero and Lamb, 2003).

The potential for bacteria and other microorganisms to be transported over long distances through the air is being increasingly examined (Burrows *et al.*, 2009). The average residence time of microorganisms in the atmosphere can range from days to weeks, long enough for cells to travel between continents. During air transport of microorganisms, they are exposed to many environmental stresses, including ultraviolet radiation, desiccation and low pH within cloud water (Burrows *et al.*, 2009). Microorganisms, such as bacteria, in aerosols are often found to be attached to mineral dust or other larger aerosol particles (Iwasaka *et al.*, 2009). It has been hypothesized that bacteria attached to larger particles are more likely to retain viability, perhaps because the particle microenvironment protects them from these environmental stresses (Iwasaka *et al.*, 2009). Though most wind-borne bacteria are typically transported <1 km from their source, dust-associated bacteria can be transported over 5000 km (Griffin *et al.*, 2003; Iwasaka *et al.*, 2009). The arrival of dust events increases the local concentration of particulate matter (PM_{10}) (particles <10 μm in size) and $PM_{2.5}$ (particles <2.5 μm in size) (Bell *et al.*, 2008; Cao *et al.*, 2014). Of particular concern are that these particles can penetrate into the lungs and the subepithelial environment (Griffin, 2007), thus leading to an increase of dust-associated potential allergen and pathogen exposure in the lungs.

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Asian dust, also called KOSA, is a meteorological phenomenon occuring mainly during the Spring, with large environmental effects, especially for eastern Asia, China, the Korean peninsula and Japan. Asian sandstorms are considered as air pollution that can affect environmental quality and may also pose a potential risk for public and environmental health, as large numbers of microorganisms may be transported along with the dust (Yang *et al.*, 2005; Griffin, 2007; Lee *et al.*, 2007). The transport of microbes by Asian dust has been reported (Shinn *et al.*, 2003; Lee *et al.*, 2009), and the presence of pathogens and allergens, with the potential to affect the health of downwind populations and ecosystems, was also revealed (Kellogg and Griffin, 2006). More than 2×10^{13} to 4×10^{16} bacterial cells km^{-2} per month were estimated to be transported to Beijing by Asian dust (Nishimura *et al.*, 2010). To identify bacteria in Asian dust, classical cultivation methods have been used to compare the differences in atmospheric colony-forming units (CFU) between normal and Asian dust days (Choi *et al.*, 1997; Iwasaka *et al.*, 2009). Choi *et al.* (1997) showed a 4.3-fold increase in the number of bacterial CFU during an Asian dust storm in Daejon (South Korea), whereas Jeon *et al.* (2010) indicated that the cultivable atmospheric bacterial population levels during sandstorms showed significant positive correlations with total suspended particles and PM_{10}. As a minority of environmental bacteria can be cultured under standard laboratory conditions (Amann *et al.*, 1995), the observed CFU counts likely represent a fraction of the actual bacterial diversity. Culture-independent methods, such as denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism, are being increasingly used in these studies (Lee *et al.*, 2009; Jeon *et al.*, 2010; Nishimura *et al.*, 2010). Using terminal restriction fragment length polymorphism analysis, Nishimura *et al.* (2010) showed that the bacterial community structures in Asian dust samples differed greatly according to the scale of the dust event. The bacterial communities from major dust events were similar to those from an arid region of China (Nishimura *et al.*, 2010). Jeon *et al.* (2010) demonstrated that the ambient air bacterial community structure was changed during Asian dust events in Seoul (South Korea) by comparing PCR-amplified 16S rDNA gene fragments using denaturing gradient gel electrophoresis-band patterns and differences in PCR-amplified 16S rDNA clone libraries between Asian dust and non-dust days (Jeon *et al.*, 2010). As particle-associated microorganisms can survive long distance transport (Griffin *et al.*, 2003; Iwasaka *et al.*, 2009), a study of sandstorm-associated microorganisms may illuminate the influence of long-range bacterial transport on downwind communities.

Here, we focused on sand-associated bacterial populations in five Asian cities in China and South Korea. In order to obtain a more complete view of sand-associated bacterial communities in these Asian sandstorm events, we used DNA pyrosequencing of 16S rDNA amplicons from total DNA extracted from sand to explore the local atmospheric bacterial composition in the presence of Asian sandstorm events. As controls, we examined local atmospheric bacterial populations that could adsorb to sterile sand, after 24 h exposure at the same locales and years, but in the absence of a sandstorm event. Sequence level comparisons were used to examine the different sandstorm and control samples, and members of several bacterial genera were found to decrease in proportion, whereas others were found to increase in proportion, during sandstorms at all sites examined, including genera that have been reported to be common in hot desert ecosystems: *Pontibacter*, *Pedobacter*, *Lysobacter*, *Planococcus* and members of the potential human pathogen *Massilia*.

MATERIALS AND METHODS

Study sites and sampling

Sampling was performed in five Asian cities over a span of 3 years. Two cities (Beijing and Taiyuan) are located in China, whereas the other three (Seoul, Gwangju and Incheon) are located in South Korea (Figure 1). Two times-autoclaved Gobi desert sand samples (25 g) (An *et al.*, 2013) were placed on sterile plastic sheets on the roof of a building in each city for 24 h in the absence of an Asian sandstorm to act as local atmospheric sand-associated bacteria controls (control samples). Sand from sandstorms was collected using sterile plastic sheets to collect settled sand at the same locations. The sampling dates and GPS site references are listed in Table 1. Sandstorms were identified by continuous monitoring of the Korean Meteorological Administration web site dedicated to identifying and following Asian dust storms: http://web.kma.go.kr/eng/weather/asiandust/intro.jsp

We sampled during periods when the PM_{10} levels were predicted to exceed 250 μg m^{-3} at the sampling sites, generally found to last for ~24 h. Samples in 2009 were taken in August, whereas samples in 2010 and 2011 were collected from February to May. Samples were named by their first letter of the sampling city (B: Beijing; S: Seoul; G: Gwangju; T: Taiyuan and I: Incheon); C or S after

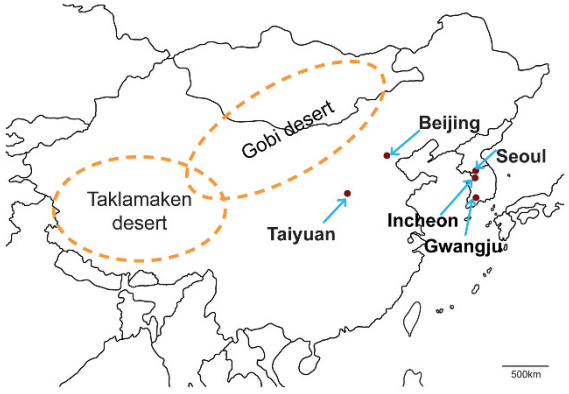

Figure 1 The location of the sampling site cities in China and South Korea, and the two major Asian deserts (Gobi and Taklamaken). The edges of the deserts are indicated by dashed yellow lines.

Table 1 Dates and location of sampling

Sample	*Date*	*Sandstorm or control*	*GPS site coordinates*
BC2009	28/08/2009	Control	39°58′45″N 116°18′57″E
SC2009	18/08/2009	Control	37°33′59″N 126°18′57″E
GC2009	21/08/2009	Control	35°09′40″N 126°56′05″E
BC2010	27/02/2010	Control	39°58′45″N 116°18′57″E
BS2010	20/05/2010	Sandstorm	39°58′45″N 116°18′57″E
GC2010	08/03/2010	Control	35°09′40″N 126°56′05″E
GS2010	19/03/2010	Sandstorm	35°09′40″N 126°56′05″E
BC2011	07/02/2011	Control	39°58′45″N 116°18′57″E
BS2011a	18/03/2011	Sandstorm	39°58′45″N 116°18′57″E
BS2011b	01/05/2011	Sandstorm	39°58′45″N 116°18′57″E
TC2011	07/02/2011	Control	37°50′02″N 112°32′34″E
TS2011a	18/03/2011	Sandstorm	37°50′02″N 112°32′34″E
TS2011b	01/05/2011	Sandstorm	37°50′02″N 112°32′34″E
GC2011	17/05/2011	Control	35°09′40″N 126°56′05″E
GS2011	13/05/2011	Sandstorm	35°09′40″N 126°56′05″E
IC2011	17/05/2011	Control	37°27′22″N 126°42′19″E
IS2011a	01/05/2011	Sandstorm	37°27′22″N 126°42′19″E
IS2011b	03/05/2011	Sandstorm	37°27′22″N 126°42′19″E

the first letter refers to control (C) or sandstorm (S), and these terminate with their sampling year. The a or b after a sampling year were added when more than one sandstorm sample was taken in the same year. After collection, samples were treated as previously described (An *et al.*, 2013), as described in detail in the following sections. Briefly, sand samples were rehydrated, total DNA was extracted, 16S rDNA genes were barcode amplified using two different thermostable DNA polymerases in several tubes to minimize PCR bias and then the DNAs were gel purified, pooled and subjected to DNA pyrosequencing.

DNA extraction

Total DNA was extracted from each sand sample as previously described (An *et al.*, 2013). From 0.5 to 2 g of sand were incubated at 30 °C with shaking for 1 h after addition of 1 ml 1/4 tryptic soy broth to both rehydrate the bacteria and minimize nutrient shock. Then, 13.5 ml extraction buffer (100 mM Tris-HCl pH 8, 100 mM Na-EDTA pH 8, 100 mM Na_2HPO_4, 1.5 M NaCl, 1% (w/v) cetyltrimethyl ammonium bromide, containing 74 μg ml^{-1} predigested Pronase plus 6.7 μg ml^{-1} RNAse A, followed by a 2-h incubation at 37 °C with mild shaking. Following this, 1.5 ml of a 20% (w/v) sodium dodecyl sulfate solution was added and incubation continued at 65 °C for a further 2 h. The supernatant fluid was collected after a 10-min centrifugation at 6000 *g* at room temperature. The pellet was extracted one more time with 4.5 ml extraction buffer plus 2% (w/v) sodium dodecyl sulfate, mixed by vortexing for 10 s, followed by incubation for 10 min at 65 °C and, after centrifugation, the supernatant fluids were pooled. The nucleic acids were extracted by the addition of an equal volume of chloroform/isoamyl alcohol (24:1) to the pooled supernatant fluids, and precipitated by the addition of 0.6 volumes of isopropanol for 1 h at room temperature, followed by centrifugation at 16 000 g for 20 min at 20 °C. The DNA pellet was washed with 70% ethanol, followed by centrifugation at 16 000 g for 5 min at 20 °C. The DNA pellets were then air-dried and resuspended in 50 μl 1/10 TE (Tris-EDTA, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA) buffer (Tolias and DuBow, 1986) at 4 °C overnight and stored at − 20 °C until use.

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) PCR

An aliquot of extracted total DNA was adjusted to a final DNA concentration of 15 ng μl^{-1} in 1/10 TE buffer using a NanoVue spectrophotometer (GE Healthcare, Buckinghamshire, UK), and verified by ethidium bromide fluorescence after electrophoresis through a 1% agarose gel in TAE (2 mM Tris-acetate pH 8, 5 mM Na-EDTA) buffer. Then, multiple 50 μl PCR reactions were performed using the universal 16S rDNA bacterial primers: 27F (*A*xxxxxx AGAGTTTGATCMTGGCTCAG) and 357R (*B*xxxxxxCTGCTGCCTYCCGTA), where *A* and *B* represent the adapters A and B for pyrosequencing using the Gold pyrosequencing reaction (GS-FLX, Roche/454 Life Sciences, CT, USA) for samples from 2009 to 2010. For the 2011 samples, primers 27F (*A*AGAGTTT GATCMTGGCTCAG) and 517R (*B*xxxxxxxxxxWTTACCGCGGCTGCTGG) were used, where *A* and *B* represent the adapters A and B for pyrosequencing using the Titanium pyrosequencing reaction (GS-FLX Titanium, Roche/454 Life Sciences). The xxxxxx or xxxxxxxxxx represent 6 or 10 nucleotide sequence tags designed for sample identification barcoding (Hamady *et al.*, 2008). PCR amplification conditions were adapted for the use of five different thermostable DNA polymerases: **I**, Phusion High-Fidelity DNA Polymerase (Finnzymes, Espoo, Finland): 98 °C for 2 min followed by 25 cycles of 98 °C for 30 s, 48 or 54 °C for 20 s and 72 °C for 12 s, and a final elongation step at 72 °C for 5 min; **II**, Pfu DNA polymerase (Fermentas, ON, Canada): 95 °C for 3 min followed by 35 cycles of 95 °C for 30 s, 48 or 54 °C for 30 s and 72 °C for 48 s, and a final elongation step at 72 °C for 5 min; **III**, High-Fidelity PCR Enzyme Mix (Fermentas): 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 48 or 54 °C for 30 s and 72 °C for 24 s, and a final elongation step at 72 °C for 5 min; **IV**, AccuPOL DNA Polymerase (Ampliqon, Herlev, Denmark): 96 °C for 2 min followed by 35–43 cycles of 96 °C for 45 s, 49 °C for 45 s and 72 °C for 1 min 12 s, and a final elongation step at 72 °C for 10 min; **V**, iProof High-Fidelity DNA Polymerase (Bio-Rad, Hercules, CA, USA): 98 °C for 30 s followed by 30 cycles of 98 °C for 10 s, 57 °C for 30 s and 72 °C for 18 s, and a final elongation step at 72 °C for 5 min. Each 50 μl PCR reaction contained 15 ng DNA, 0.1 μM of each primer (Sigma-Aldrich, St Louis, MO, USA), 0.2 mM dNTP mix (Fermentas), 1.25 units polymerase for **I** and **II**, 2 units polymerase for **III**, 2.5 units polymerase for **IV** and 1 unit polymerase for **V** using the buffers supplied with each polymerase. Each DNA sample was subjected to 5–10 PCR reactions per thermostable DNA polymerase, and two different polymerases were used per sample to minimize PCR bias. The resultant PCR products were pooled and loaded on a 1% agarose gel in TAE buffer (An *et al.*, 2013). After electrophoreses and DNA visualization by ethidium bromide staining and long-wave ultraviolet light illumination, the 16S amplified DNA fragment-containing regions were cut from the gel and purified using the NucleoSpin Extract II kit (Macherey-Nagel, North Rhine-Westphalia, Germany) according to the manufacturers' instructions. Fifty nanograms of PCR products from each sample were mixed for pyrosequencing.

bTEFAP FLX pyrosequencing

Pyrosequencing was performed using a Roche/454 FLX Pyrosequencer (Microsynth AG, Balgach, Switzerland). The sequences obtained for each sample were grouped according to the tag used, and the average sequence was found to be 227 nucleotides in length for those using the Gold reaction and 503 nucleotides in length for the samples using the Titanium reaction, after removal of the tags.

bTEFAP sequence processing pipeline and data analyses

The sequences were selected by their length (>150 bases) and their quality score (90% of nucleotides with a quality score >25) using the Greengenes website (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi). Then, sequences with more than two errors in the primer or more than one ambiguous base were removed using the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu/index.jsp).

The remaining sequences were then classified to the genus level using RDP-II-naive Bayesian classifier (Liu *et al.*, 2007; Wang *et al.*, 2007) with the confidence threshold set to 50% for Gold reaction samples and 80% for Titanium reaction samples. The sequences classified as chloroplast were eliminated using 'FASTA Sequence Selection' in RDP's pyro site. The non classified sequences were used for a further BLAST against the Genbank database to detect potential mitochondrial DNA sequences. In order to compare the estimations for bacterial population richness and diversity, we also normalized the sequence numbers for each sample by randomly selecting 3400 sequences from each sample from the fasta file using a perl script called 'selector.pl' in Pangea (Giongo *et al.*, 2010). The Chao1 estimator and Shannon indices were calculated on the RDPII-pyro site, whereas the Bray–Curtis index was calculated using SPADE (http://chao.stat.nthu.edu.tw) to build the unweighted pair group method with arithmetic mean (UPGMA) clustering tree. The similarity level among the different operational taxonomic units (OTUs) was fixed at 97%. We calculated the *P*-value of the chi-square test for the bacterial populations from each pair of samples using R software (http://www.r-project.org/). Principal component analyses (PCA) on the relative proportion of phyla or OTUs among the samples were performed using the ade-4 package (Chessel *et al.*, 2004) adapted in R. All sequence data have been deposited in the MG-RAST (http://metagenomics.anl.gov/metagenomics.cgi?page=Home) database under numbers 4487160.3–4487161.3, 4487162.3–4487166.3, 4487168.3–4487172.3, 4487174.3–4487177.3, 4487179.3–4487181.3.

RESULTS

DNA sequence quality assessment

We collected nine different dust/sand samples, on sterile plastic sheets, that fell during Asian sandstorm events in five Asian cities (Beijing and Taiyuan in China; Gwangju, Incheon and Seoul in South Korea) in 2010 and 2011 (Figure 1). As controls, we prepared sterile sand from the Gobi desert and allowed local atmospheric bacteria (in the same locations used for sandstorm collections) to adhere to the sand, spread on sterile plastic sheets, for 24 h in the absence of a sandstorm (nine different samples from 2009 to 2011). After total DNA extraction from each of the collected sand samples, 16S rDNA PCR amplification and DNA pyrosequencing, a total of 507 778 sequences were obtained. After quality control checks and sequence elimination using the

Greengenes and RDPII pyrosequencing pipelines, followed by B2C2 chimera sequence removal, a total of 361 748 sequences remained. The average sequence length after the quality control steps was 229 nt for pyrosequencing Gold reactions and 373 nt for the pyrosequencing Titanium reactions. Chloroplast sequences were detected (and removed) in 13 of 18 samples, with their proportion varying between 0.04 and 4.2%. As mitochondrial 16S rDNA sequences would be revealed as unclassified sequences using the RDP classifier, we examined the unclassified sequences in Genbank using BLAST and found that the proportion of mitochondrial DNA sequences represented $<0.01\%$ of the sequences per sample.

Richness and diversity in sand-associated bacterial populations

We observed that the detectable OTU numbers varied from 321 to 4026 per sample. Nonetheless, ChaoI estimations suggest that we were able to identify from 50 to 90% of the total OTU population (Table 2). In total, bacteria from 951 different genera were detected in our samples. As shown in Table 2, only the control samples from Gwangju (2010) and Incheon (2011) contained fewer than 1000 OTUs, as calculated using Chao1, though the Gwuangju 2010 sample had 3505 sequences, whereas the Incheon 2011 sample had 23 466 sequences, a sevenfold difference. Normalizing the sequences to 3400 per sample did lower the estimated population richness (Chao1), whereas the Shannon diversity index remained relatively stable. It is interesting to note that during non-sandstorm event days, the samples from China displayed a significantly higher bacterial population richness (Chao1) than the samples from South Korea ($P=0.03$). The bacterial OTU richness and diversity from the same sampling site varied among the different years, as well as between non-sandstorm controls versus sandstorm samples. No consistent trends were observed when comparing the richness and diversity between the control samples and sandstorm samples at each sampling site over the 3 years.

Sequence level comparisons and bacterial population composition

Figure 2 shows the composition of the bacterial populations of the different samples at the phylum level. All the samples were dominated (~90% of the sequences) by members belonging to four phyla: *Proteobacteria*, *Bacteriodetes*, *Actinobacteria* and *Firmicutes*, although no obvious (for example, control versus sandstorm) differences were discernible. In order to reveal any underlying tendencies in the composition of the different bacterial populations, a UPGMA clustering tree (Figure 3) was generated with the data of pairwise Bray–Curtis indices calculated using SPADE (sequence similarity level at 97%). The 18 samples were found to contain distinct bacterial communities. Nonetheless, the control samples were found to be best clustered by sampling year, as were the sandstorm samples. As the temporal variability of air-borne bacteria has been previously reported (Bowers *et al.*, 2012), PCA were performed only for samples from the same season, and only on samples from 2010 and 2011, as these have both sandstorm and control samples. PCA, using the sequence-normalized data, were generated using the ade-4 package of R (Figure 4). We observed that samples were best able to be clustered by sampling year, rather than sampling condition (control versus sandstorm) or sampling site, as observed using UPGMA clustering (Figure 3). PCA at the phylum level explained close to half (46.3%) of the total variance. The large difference observed between GS2010 and the other samples was due to the higher abundance of members belonging to the phyla *Bacteroidetes*, *Cyanobacteria*, *Fibrobacteres*, *Deferribacteres,* OD1 and WS3. The differences among samples from 2010 versus samples from 2011 at the OTU/species level were more difficult to discern. We observed that samples from 2010 contained more OTUs belonging to *Delftia sp.* and *Acinetobacter sp.*, whereas samples from 2011 contained more OTUs belonging to *Planococcus sp.*, *Psychrobacter sp.*, *Methylobacterium sp.*, *Herbaspirillum sp.*, *Rubellimicriobium sp.* and *Microvirga sp.*

We were able to discern nine genera whose members increased in sandstorm samples versus the controls (Table 3), with the exception of the samples from Gwangju in 2011. The reason for this exception will

Table 2 Number of processed pyrosequencing sequences, OTU richness and diversity for each sample

Sample	*High-quality sequences*					*Normalized sequences*[a]		
	Number of sequences	*OTUs*	*Chao1*	*% Coverage*	*Shannon index*	*OTUs*	*Chao1*	*Shannon index*
BC2009	10 895	2353	3517	66.9	6.82	1179	2229	6.41
GC2009	4545	791	1203	65.8	5.37	683	1108	5.34
SC2009	8383	1315	2086	63.1	5.75	776	1381	5.59
BC2010	3386	603	1170	51.5	4.35	603	1170	4.35
GC2010	3505	321	474	67.7	2.90	315	473	2.90
BC2011	31 959	3419	4247	80.5	6.77	975	1496	6.05
TC2011	21 603	3244	4452	72.9	6.84	1074	1784	6.18
GC2011	27 054	1915	2671	71.7	5.48	550	942	5.01
IC2011	23 466	506	554	91.4	4.93	340	381	4.74
BS2010	6462	649	1133	57.3	4.70	434	711	4.45
GS2010	3837	1227	2036	60.3	6.39	1135	1938	6.35
BS2011a	22 323	3025	4297	70.4	6.37	872	1460	5.61
BS2011b	17 960	2676	3644	73.4	6.49	966	1733	5.91
TS2011a	49 755	4026	5556	72.5	6.19	748	1221	5.28
TS2011b	20 139	2092	2841	73.6	5.59	683	1045	5.00
IS2011a	22 047	2666	3507	76.0	6.55	897	1378	5.99
IS2011b	47 026	2637	2990	88.2	6.07	790	1332	5.50
GS2011	22 893	1012	1365	74.1	4.31	357	510	4.00

Abbreviation: OTU, operational taxonomic unit.
[a]The sequence numbers of each sample were randomly normalized to 3400 using *Selector.pl* in Pangea.

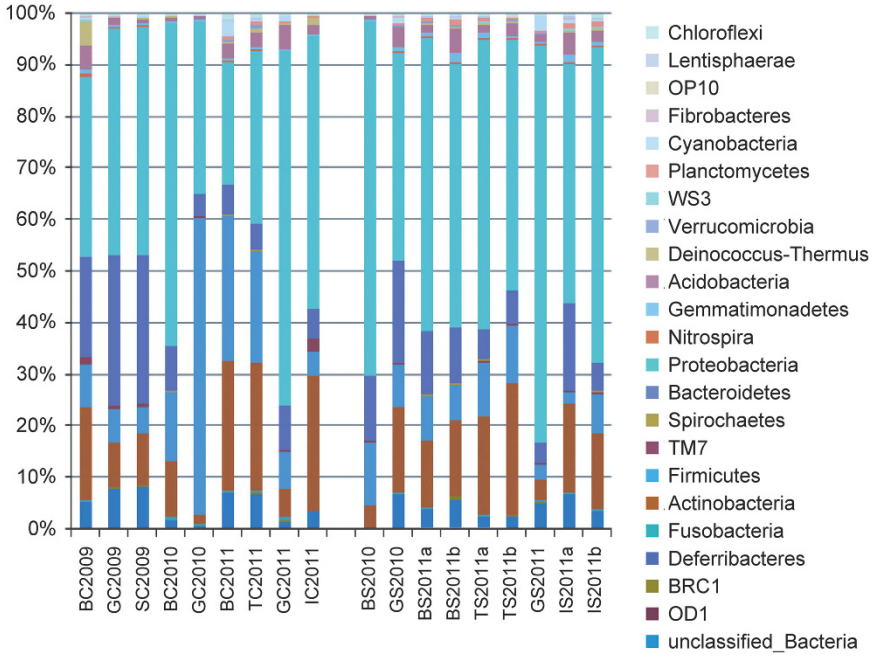

Figure 2 The relative composition of phyla of the sandstorm and control sand-associated bacterial populations. The samples on the left represent the sand-associated controls from non-sandstorm days, while those on the right represent sand-associated bacteria from sandstorm samples. See the Materials and methods for the naming scheme of the samples.

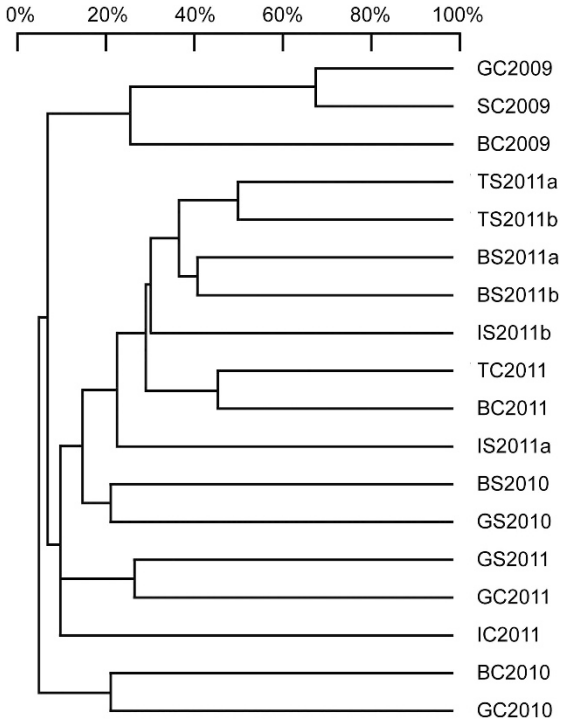

Figure 3 A UPGMA clustering tree of the sandstorm and control sand-associated bacterial populations using a Bray–Curtis similarity index (97% cutoff) among samples, as described in the Materials and methods. Each sample was normalized to 3400 sequences.

be discussed later. We also detected OTU members from five genera (grouping members of the closely related *Escherichia* and *Shigella* genera) whose members were always proportionally reduced in sandstorm samples, versus the controls (Table 3).

DISCUSSION

Richness and diversity of sand-associated bacterial populations

Increasing evidence has shown that the surface sands of Asian deserts, such as the Gobi and Taklamaken deserts, rather than being barren extreme environments, can contain diverse bacterial populations (An *et al.*, 2013). It is thus not surprising that when desert soil is mobilized into the atmosphere, the concentrations of atmospheric sand-associated microorganisms can increase in the downwind regions (Griffin, 2007; Cao *et al.*, 2014). Here, we explored the richness and diversity of sand-associated bacterial populations in gravity-collected sand (and dust) during sandstorms, as well as sterile sand-adsorbed local atmospheric bacteria, using pyrosequencing of PCR-amplified 16S rDNA fragments from total DNA extracted from samples taken during Asian sandstorms, as well as controls (sterile sand-associated bacteria). We used gravity collection, as opposed to impactor or aspiration-mediated methods (Cao *et al.*, 2014), in order to more accurately reflect environmental and human exposures to these particles. However, it is important to state here that the control samples will include both dust and sterile sand-associated bacteria, whereas sandstorm samples will include dust, transported sand and non-desert bacteria that adsorb to these during atmospheric transport. After trimming and removal of low-quality DNA sequences, we examined both total and sequence-normalized (3400 sequences) populations in order to make certain that sequence number differences were not markedly affecting our results (An *et al.*, 2013).

Bacterial population composition

We sampled in Beijing and Gwangju each year from 2009 (only controls of sand-associated bacteria in the absence of a sandstorm) through to 2011 (sandstorm and control samples), with Incheon (South Korea) and Taiyuan (China) also sampled in 2011. As the PCA and UPGMA analyses showed, the samples did not appear to cluster by sampling site. These results suggest that variations in the structure of the sand-associated bacterial communities may be more influenced

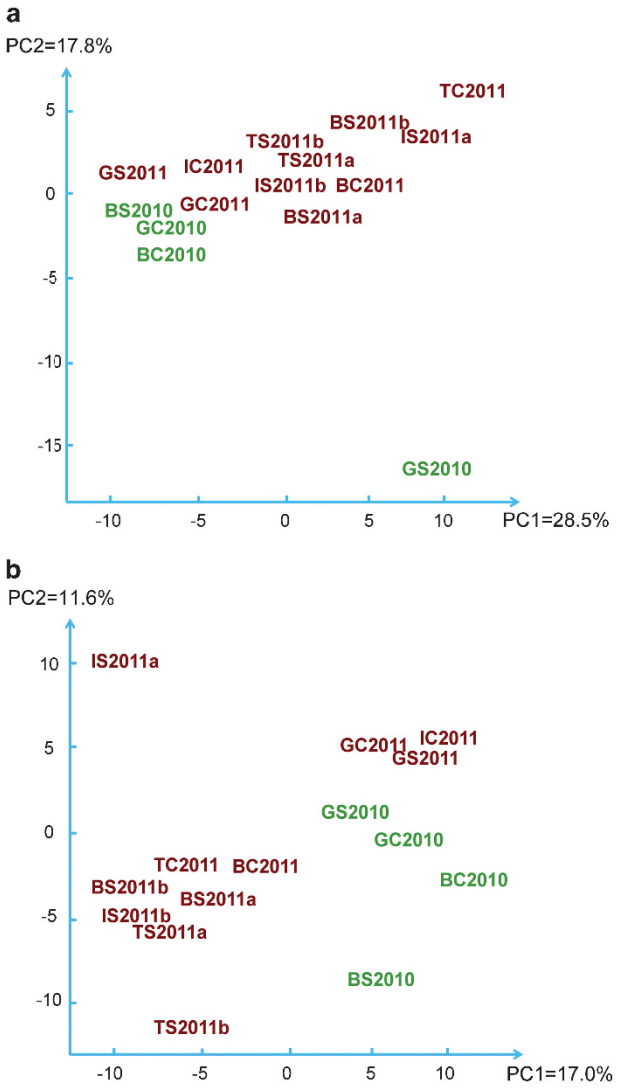

Figure 4 Normed PCA using phyla composition data (**a**) or OTU composition (**b**) data generated by R. Red letters are used to represent samples from 2011, whereas green letters are used to represent samples from 2010. See the Materials and methods for the naming scheme of the samples.

by time scale than by geographic proximity at the locations studied and are in agreement with other studies dealing with the annual variations in the diversity of atmospheric bacteria (Maron *et al.*, 2005; Fahlgren *et al.*, 2010). As the samples were clustered by their sampling year, we can hypothesize that an important variable in the differences in atmospheric bacterial populations may be due to changeability in the prevailing winds in each year (Cao *et al.*, 2014). However, besides local meteorological conditions, the spatial variability of air-borne bacterial richness has been reported as being influenced by several different environmental factors, including land-use types and the distance from the sea (Bowers *et al.*, 2011, 2012). The exception of the 2011 Gwangju sample may be due to the sampling time of the control sample, which was 4 days after the passage of sandstorm (see Table 1), suggesting that the effect of local atmospheric bacteria modifications of atmospheric particle-associated bacterial composition by sandstorms may remain for several days after the major event has passed.

Table 3 The sandstorm-associated bacterial genera increased or reduced, versus the controls, at each site in 2010/2011 (excluding the Gwangju 2011 sample)

	Beijing 2010	*Gwangju 2010*	*Beijing 2011*	*Taiyuan 2011*	*Incheon 2011*
Increase in sandstorm					
Massilia	6276%	771%	1434%	89%	2843%
Planococcus	71%	539%	99%	298%	—
Carnobacterium	251%	37%	105%	546%	—
Planomicrobium	105%	—	57%	343%	—
Pontibacter	2258%	—	141%	53%	403%
Pedobacter	1891%	296%	1514%	205%	1391%
Lysobacter	—	—	97%	85%	88%
Sanguibacter	179%	—	55%	634%	—
Ohtaekwangia	—	—	268%	579%	—
Decrease in sandstorm					
Escherichia, Shigella	−69%	−95%	−56%	−89%	−97%
Acinetobacter	−82%	−32%	−40%	−37%	−72%
Propionibacterium	−100%	−100%	−45%	−69%	−91%
Corynebacterium	−95%	−85%	−24%	−40%	−76%
Streptococcus	−96%	−96%	−68%	−67%	−91%

— Indicates that members of the genus were non-detectable in non-sandstorm (control) samples.

Dominant bacteria

Previous studies, using mostly cultivation and/or DNA cloning and sequencing techniques, found that the predominant sand-associated bacterial groups in sandstorms were Gram-positive (such as members of the genus *Bacillus*) bacteria (Larsson *et al.*, 1999; Lee *et al.*, 2009), presumably because they can form desiccation and ultraviolet-resistant spores. Here, we found that Gram-negative bacteria, particularly members belonging to the phylum *Proteobacteria,* appear to be the predominant bacteria in particle-associated bacterial populations in the Asian sandstorm samples. In addition to the use of cultivation-independent DNA pyrosequencing of PCR-amplified 16S rDNA to assess the total bacterial populations, these differences may be due to differences in the sandstorm desert source bacterial populations (An *et al.*, 2013; Prestel *et al.*, 2013). As our samples could be grouped using UPGMA and PCA, it is likely that the origin of the sandstorms may be important in explaining the differences observed among different studies, in addition to wind sources and trajectories.

Genus level population modifications

Previous studies on the detection of bacteria in sandstorms did not always take into consideration the influence of locally present atmospheric bacteria in the absence of a sandstorm (Fahlgren *et al.*, 2010). We thus obtained sand/dust-associated total DNA and analyzed the bacterial communities during both non-sandstorm and sandstorm days via DNA pyrosequencing and bioinformatics analyses. We found members from nine genera whose proportions increased in sandstorm event days (Table 3).

Members of the genus *Massilia* appear to be very abundant in sandstorm samples. However, they were not as readily abundant in the control samples, except for the Gwangju 2011 control sample, which was collected 4 days after a sandstorm. It is interesting to note that members belonging to the genus *Massilia* have been detected in desert environments (Chanal *et al.*, 2006; An *et al.*, 2013) and sandstorm samples (Griffin, 2007). That the detectable 16S rDNA sequences have

⩾96% sequence similarity with that from the species *Massilia timonae*, isolated from patients (La Scola *et al.*, 1998) and reported to cause wound infections (Van Craenenbroeck *et al.*, 2011), suggests a potential *Massilia* infection risk for humans in Asian sandstorm downwind regions, although it should be emphasized here that most of the species belonging to the genus *Massilia* are not human pathogens.

Among the nine genera whose proportions increased during Asian sandstorms, members of the genera *Pontibacter*, *Pedobacter* and *Lysobacter* were also detected in the desert soils of China (Zhou *et al.*, 2007; Tang *et al.*, 2010; An *et al.*, 2013), whereas members of the genus *Planococcus* have been isolated from African desert dust events (Griffin, 2007). These results suggest that members of these bacterial genera may be common in hot desert environments and subjected to atmospheric dispersal.

Potential health threats

In Seoul, increases of daily deaths due to Asian sandstorm events have been reported, especially for persons with advanced cardiovascular and respiratory disease (Kwon *et al.*, 2002; Lee *et al.*, 2007). In Taipei City of Taiwan, the rate of clinic visits for children increased 2.54% for preschool children and 5.03% for school-age children after Asian sandstorms (Chien *et al.*, 2012). These results were often presumed to be due to dust mineral and chemical irritant/allergen penetration into the lungs. However, exposure to microbial and microbial-component-laden (lipopolysaccharide of Gram-negative cells) air-borne soils can also cause respiratory stress (Larsson *et al.*, 1999).

After long-range transport, the diversity of bacteria deposited by sandstorms may have a significant role in ecosystem and human health and warrants further research in this emerging field (Hwang *et al.*, 2005; Yang *et al.*, 2005). In the future, more samples should be taken for spatial and temporal comparisons and studies should extend to the study of the domain of the Archeae, as well as eukaryotic microbes and viruses.

DATA ARCHIVING

All sequences data have been deposited in the MG-RAST database (http://metagenomics.anl.gov/metagenomics.cgi?page=Home) under number 4487160.3–4487161.3, 4487162.3–4487166.3, 4487168.3–4487172.3, 4487174.3–4487177.3, 4487179.3–4487181.3.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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