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journal or publication title	Air Quality, Atmosphere and Health
volume	2
number	4
page range	195-202
year	2009-01-01
URL	http://hdl.handle.net/2297/27318

Dustborne Microorganisms in the atmosphere over an Asian dust source region, Dunhuang

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Abstract

The dust event injects microorganisms into the atmosphere and could facilitate the dispersal of biological particles affecting leeward ecosystem and human health. In this study, the dustborne microorganisms in the atmosphere over the Taklimakan Desert, Asian dust source, were identified by culture-independent method. Dusts were collected using a balloon at about 800 m above the ground in an Asian dust source region, Dunhuang. After DNA were directly extracted from the dusts collected filters, 16S and 18S rRNA genes of microorganisms were amplified, cloned and sequenced. The rDNA sequence data indicated that dust particles include fungi closely related to *Rickenella fibula*, *Ceriporiopsis gilvescens* and bacteria belonging to the genus *Brevibacillus*, *Staphylococcus*, *Rhodococcus*, *Delftia*, *Pseudomonas* and *Agrobacterium tumefaciens*. These results suggest that dust particles in the atmosphere over Dunhuang could carry these many fungi and bacteria and might play a significant role in leeward ecosystem.

Key Words: Asian dust, dustborne, microorganism, bacteria, fungi

Introduction

Asian dust from China is blown eastward across Korea, Japan and the Pacific Ocean (Duce et al. 1980; Tsunogai et al. 1982). The global scale transport of Asian dust has been demonstrated by the chemical and radiological analysis of deposited dust in Hawaiian soil (Kennedy et al. 1998; Chadwick et al. 1999), the Greenland ice core (Biscaye et al. 1997) and St. Elias mountain, Canada (Zdanowicz et al. 2006). The major sources of Asian dusts are the Taklimakan Desert, Loess Plateau, and Gobi Deserts. However, dust events from the sources have different characteristics and contributions to downwind regions. Sun et al. (2001) suggested that dusts from the Gobi Deserts of Mongolia and northern China can only be entrained to an elevation of < 3000 m and deposited mainly in the proximal region in most cases. On the other hand, dust particles from Taklimakan Desert can be entrained to an elevation of > 5000 m and then transported over long range by the westerlies, since area to the north, west, and south of the Taklimakan Desert are surrounded by high mountains (average elevation > 5000 m) and open area of the desert exists only in the east margin. Dust particles, which cannot move up over the surrounding mountains, were well mixed about from several hundred meters to 6 kilometers above the ground at Taklimakan Desert, Tarimu basin (Iwasaka et al. 2003, Yamada et al. 2005). Kurosaki et al. (2005) indicated that there are frequent floating dusts in the Taklimakan Desert even in summer because of the steep topographical surroundings. The floating dusts are rarely observed in the Gobi Desert and Loess Plateau. By lidar measurements, the weak dusts were detected in the free troposphere over Japan during periods with no evident dust outbreak or even in seasons other than spring (Iwasaka et al. 1988, Sakai et al.

2000). Some investigators suggested that weak Asian dust events make possible contribution of biogeochemical cycle of the land, atmosphere, and ocean (Iwasaka et al. 2003; Matsuki et al. 2003; Yamada et al. 2005).

In recent years, many epidemiological studies have shown that Asian dust events are associated with an increase in risk of mortality and patients of cardiovascular and respiratory illness in Korea (Kwon et al. 2002; Park et al. 2005; Lee et al. 2007) and Taiwan (Chen et al. 2004 and 2005; Chan et al. 2008).

There is also a report that lipopolysaccharide and β -glucan have been detected in Asian dust particles (Ichinose et al. 2005). Lipopolysaccharide is the major component of outer membrane of Gram-negative bacteria (Nikaido et al. 1985) and induces or aggravates a variety of respiratory disease (Tulic et al. 2000; Wu et al. 2002). β -glucan is a component of cell wall of fungi (Shaun et al. 2006) and relates to respiratory tract inflammation (Vassallo et al. 2000; Hahn et al. 2003).

In fact, four research groups have detected bacteria and fungi in Asian dust by culture-based or microscopic analyses, in the last decade (Choi et al. 1997; Yeo et al. 2002; Wu et al. 2004; Ho et al. 2005). Dustborne microorganisms aerosolized by African desert winds have been also investigated by culture-based methods (Griffin et al. 2001, 2003, 2006 and 2007; Kellogg et al. 2004; Prospero et al. 2005).

These desert dust events can facilitate long-distance dispersal of these dustborne microorganisms. The microorganisms should be considered as an important factor which affects air quality, human health and downwind ecosystem.

In conventional culture-based method, however, only 1 % of environmental microorganisms is culturable on

any given medium (Torsvik et al. 1990). That is, about 99% of the total microorganisms in environment are unable to be detected or analyzed under laboratory conditions. It is essential to clarify the all dustborne microorganisms, and the role and dynamics of microbial communities in air quality.

On the other hand, DNA isolated directly from dusts could provide genetic information of the unculturable microorganisms. In microbiology of soil and aquatic environments Remarkable advances have been made recently in microbiology of soil and aquatic environments using culture-independent, molecular approaches (Rondon et al. 2000; Moon-van der Staay et al. 2001; Lopez-Garcia et al. 2001; Dawson et al. 2002; Venter et al. 2003).

In this study, we investigated the dustborne microorganisms in the atmosphere over the Dunhuang, which is located in east open area of the Tarimu basin (Taklimakan Desert), by culture-independent, metagenomic rDNA analysis.

Materials and method

Dusts samplings were performed in Dunhuang (40°10'00"N, 94°40'60"E), Gansu Province, China, on August 17, 2007. Dunhuang city is located in the eastern part of Taklimakan Desert. Dust event was not observed on our sampling day by Meteorological Bureau of Dunhuang city. The wind was calm at the ground on our sampling day. Airborne dusts were collected on 0.45 µm pore-size filters by a vacuum pump using a balloon, at 800 m and 10 m above the ground. Before the sampling, 0.45 µm filters were autoclaved and set to bioaerosol sampler aseptically. Samplings were performed by remote control, when the balloon had reached the target altitude (Yamada et al. 2005). It was estimated that the Asian dusts in about 0.7 m³ of atmospheric air was collected on filter, by using the vacuum pump for 1 hour. The particle counter (KR-12A, RION CO., Ltd.) and thermohygrometer (EX-501, EMPEX Instruments, Inc.) were used with the balloon during the dusts sampling.

DNA was extracted from dusts on the collected filter using cell wall lytic enzyme, lysozyme and proteinase K (Sigma-Aldrich). 16S rDNA for prokaryote was amplified by PCR as described by Weisburg et al. 18S rDNA for eukaryote was amplified by PCR using primer F1 (5'-TGGTTGATCCTGCCAGAGG-3') and R1 (5'-GGCTACCTTGTTACGACTT-3'). Each PCR reaction mixture (vol. 20 µl) included the following: 4 µl of 5 x Buffer, 1.6 µl of 10 x dNTP (2.5 mM each, dATP, dCTP, dGTP, dTTP), 0.2 µl of each primers (20 mM), 12.8 µl of sterile deionized H₂O, 1 U of PrimeSTAR DNA polymerase (TAKARA BIO INC.), 1 µl of DNA

(~30 ng). Thermal cycler (Dice, TAKARA BIO INC.) was used under the following conditions for amplification: initial 2 min denaturation at 98 °C; 35 cycles – 10 sec. denaturation at 98 °C, 10 sec. annealing at 54 °C, and 1.5 min extension at 72 °C; final 3 min extension at 72 °C.

rDNA clone library was constructed the following procedure, as described previously (Kakikawa et al. 2002); Amplified 16S and 18S rDNA (1.5 kb and 1.7 kb, respectively) were cloned by ligation to the *Hinc* II site of plasmid pUC119 and introduced into *Esheria coli* JM109 by electroporation (BIO-RAD Lab.). Inserted rDNA clones were screened by colony PCR. Then, a total of 685 clones with a rDNA insert was divided into 29 representative variants by RFLP (Restriction Fragment Length Polymorphism) analysis using restriction enzymes *Rsa* I and *Alu* I (TAKARA BIO INC.).

The DNA sequences of cloned rDNA were determined by genetic analyzer (Applied Biosystems) and the related species of dustborne microorganisms were searched by BLAST analysis (<http://www.ncbi.nlm.nih.gov/BLAST/>) to DNA databases (GenBank/EMBL/DDBJ).

These sequence data of dustborne microorganisms have been submitted to the GenBank database under accession numbers AB451535 to AB451542 for the bacteria, and AB451531 to AB451533 for the fungi.

Results and discussion

Dusts sampling

Airborne dusts were collected on 0.45 μm pore-size filter using a balloon with filtration system at 800 m and 10 m above the ground in Dunhuang (40°10'00"N, 94°40'60"E), Aug. 2007. The dusts above Dunhuang city have been known as one of large source areas of Asian dust particles, since Dunhuang city is located at east open area of the Taklimakan Desert surrounded by high mountains (average elevation > 5000 m).

During dusts sampling at 800 m above the ground, mean temperature and mean relative humidity were 13.6°C and 94.1%, respectively. At the same time, mean temperature and mean relative humidity on the ground were 23.0°C and 63.4%, respectively.

At 800 m above the ground, the particles with diameter 0.3 μm showed number concentration of 50,000 / L and formed about 75.5% of the total particles on the sampling site (Fig. 1a). The particles with diameters 0.5 - 2.0 μm and larger than 2.0 μm showed number concentration of 15,000 / L (23%) and 1,000 / L (1.5%), respectively. At 10 m above ground, the particle concentration with size of a diameter 0.3 μm was higher than that of 800 m above the ground, and changes in the number concentration were seen during sampling (Fig. 1b). Number concentration of particles with size ranges of 0.5 - 2.0 μm and > 2.0 μm at 10 m above the ground show 15,000 / L (17.3%) and 1,100 / L (1.3%), respectively. These results indicate that the number concentration of particles at 10 m above the ground was about the same as that of 800 m above the ground. However, particle concentration at 10 m height had much variation during sampling time and that at 800 m

height was relatively stable for a long time. It is considered that the change of particle concentration at 10 m height was large compared with that of 800 m height in order to disturb the air near the ground by the thermal and frictional effect of earth surface.

By vacuum performance, it was estimated that the dusts particles ($> 0.5 \mu\text{m}$) in about 0.7 m^3 atmosphere air 800 m above ground were collected on $0.45 \mu\text{m}$ pore-size filter. Thus, it can be estimated that the 1.12×10^7 dusts particles (1.05×10^7 particles with diameter $0.5 - 2.0 \mu\text{m}$ and 0.07×10^7 particles with diameter larger than $2.0 \mu\text{m}$) were collected on the filter in this sampling.

DNA contained in Asian dusts

DNA was extracted from dust particles on the filters with cell wall lytic enzyme, lysozyme and proteinase K. The amount of DNA was about $0.1 \mu\text{g}$ from the filter of 800 m above the ground, and about $0.2 \mu\text{g}$ from the filter of 10 m height above the ground.

It was estimated that the number of microorganisms at 800 m above the ground was $\sim 2 \times 10^6$ in the 0.7 m^3 atmosphere air, because $1 \mu\text{g}$ of DNA contains 2×10^7 molecules of bacterial or fungi genome. In 10 m above the ground, it was estimated $\sim 4 \times 10^6$ microorganisms in the 0.7 m^3 atmosphere air. It remains to be seen whether these microorganisms were suspended freely or attached to dust particles.

16S rDNA for bacteria (1.5 kbp) and 18S rDNA for fungi (1.6 kbp) were amplified by PCR with using universal primers as shown in Fig. 2. The data indicate that the dust particles from heights of 800 m and 10 m include both bacteria and fungi. Since the detected DNA band was rDNA mixture from various bacteria and

fungi, rDNA clone library was constructed by using plasmid vector pUC119. Then clones of 16S rDNA and 18S rDNA were selected by RFLP analysis and sequenced. The related species of dustborne microorganisms were identified by BLAST search to DNA database.

Dustborne microorganisms of 800 m above the ground in Dunhuang

By nearly complete 18S rDNA sequence analysis, it was indicated that closest species to *Rickenella fibula* (AY771599) and *Ceriporiopsis gilvescens* (AY219403) were included in the atmosphere 800 m above the ground in Dunhuang (Table 1). These species are known as spore-forming fungi. The clone DHUP10 fungus shows a high DNA homology of 99.6% to an uncultured fungus in soil (Moon-van der Staay 2006, AM114819). In DNA database, however, there was no sequence similar to two 18S rDNA species derived from the dust particles in atmosphere air of 800 m height. This result suggests the existence of undiscovered eukaryotic species. Recent studies of aquatic environments show unexpected eukaryotic diversity, by similar molecular approach (Moon-van der Staay et al. 2001; Lopez-Garcia et al. 2001). It is possible that many novel microorganisms markedly resistant to ultraviolet light or desiccation exist in atmosphere.

16S rDNA data indicated that dust particles from 800 m height in Dunhuang included bacteria closely related to *Brevibacillus sp.* (AJ313027, 99.8%), *Rhodococcus sp.* (DQ285075, 99.8%), *Delftia sp.* (EU880508, 100%), *Pseudomonas sp.* (AM411620, 99.8%) and *Agrobacterium tumefaciens* (EU592041, 99.9%). The clone DHUP7, DHUP34 and DHUP66 bacteria show a high DNA homology to uncultured *Staphylococcus sp.* (EU660426, 99.4%), uncultured *Pseudomonas sp.* (DQ088809, 99.7%) and uncultured bacterium (AY958912,

99.7%) isolated by culture-independent method.

Three bacterial species of *Brevibacillus*, *Staphylococcus* and *Rhodococcus*, belong to Gram-positive bacteria.

Brevibacillus sp. is a spore-former, whereas *Rhodococcus sp.* has a feature of photosynthetic bacteria. Three species of *Delftia*, *Pseudomonas* and *Agrobacterium tumefaciens* belong to Gram-negative bacteria, which have lipopolysaccharide and can cause and aggravate respiratory diseases. *Agrobacterium tumefaciens* was typically found in soil and has been known as a plant pathogen.

It should be noted that clone DHUP23 is closely related to *Delftia sp.* (EU880508) isolated from river estuary sediment in southern China. Clone DHUP49 is also closely related to *Pseudomonas sp.* (AM411620) isolated from deep sea. These ocean bacteria, *Delftia sp.* (EU880508) and *Pseudomonas sp.* (AM411620) might be of Asian desert origin and have been transported to the sea by desert wind.

These results on dustborne microorganisms of 800 m above the ground indicate that dusts contain at least eight bacterial and three fungal species including soil bacterium, spore-forming bacterium and plant pathogenic fungi. Thus these dustborne bacteria and fungi have possibilities of affecting downwind ecosystem.

On the other hand, Maki et al. (2008) detected the dust particles with attached bacteria in the atmosphere in summer over same location, Dunhuang using an epifluorescence microscope. Maki et al. analyzed the bacterial community in the dusts by using the culture-based and denaturing gradient gel electrophoresis (DGGE) methods, and detected a halobacterial community composed of members of the genus *Bacillus* and *staphylococcus*. Yeo et al. (2002) reported that four fungi of *Aspergillus*, *Basipetospora*, *Fusarium* and *Penicillium* were detected from suspended particulate matter (SPM) samples taken at Seosan, Korea.

According to Wu et al. (2004) and Ho et al. (2005), fungal spores such as *Cladsporium*, *Ganderma*, *Arthrium*, *Cercospora*, *Stemphylium*, *Pithomyces*, *Periconia*, *Alternaria*, *Botrytis* and *Nigrospora*, had significantly higher number concentrations in Taiwan, during Asian dust event.

From the results of our and other research group, it has been revealed that microorganisms could be transported by airborne dust, although these have differences in the genera of bacteria or fungi. The differences might have been caused by another methods, or dust sampling points.

Further studies should examine the seasonal variation, transportation change of dustborne microorganisms from dust source region to downwind area and the effects on ecosystem as air quality.

Dustborne microorganisms of the ground in Dunhuang

18S rDNA data show that dust particles of 10 m above the ground in Dunhuang contain fungi closely related to *Henningsomyces sp.*, *Athelia bombacina*, *Tulasnella sp.* *Nyssopsora echinata*, *Wallemia sp.* and seem to contain plant pollen of *Beta vulgaris*, *Caprifoliaceae* and *Panax notoginseng* (data not shown). *Nyssopsora echinata* is known as a plant pathogen.

Dustborne bacteria on the ground in Dunhung were related to *Arthrobacter sp.* *Ornithinimicrobium sp.*, *Rubellimicrobium thermophilum*, *Friedmanniella capsulala*, *Geodermatophilus sp.*, *Cellulomonas sp.*, *Promicromonospora sp.* and *Cryocola antiquus*.

In spite of having collected at the same location, dustborne microorganism species of 800 m height did not consist with that of 10 m height. Dust particles of 800 m above the ground might have been transported from

central and west Taklimakan Desert by west wind. There might be microorganisms which can soar up in the air with dust and, needless to say, microorganisms which cannot soar.

Conclusions

Dust particles in the atmosphere over Dunhuang city, China, were collected on Aug. 2007. Dustborne microorganisms in the atmosphere over the Dunhuang city were investigated by culture-independent rDNA Analysis. Three fungal species closely related with *Rickenella fibula* (AY771599), *Ceriporiopsis gilvescens* (AY219403), uncultured fungus (AM114819) and previously unknown 2 eukaryotic species were detected in dusts from 800 m above the ground. Eight bacterial species belonging to the genus *Brevibacillus* (AJ313027), *Staphylococcus* (EU660426), *Rhodococcus* (DQ285075), *Delftia* (EU880508), *Pseudomonas* (DQ088809, AM411620), *Agrobacterium* (EU592042) and uncultured bacteria (AY958912) were found in dusts from 800 m above the ground. This bacterial community contains plant pathogen, soil bacteria and spore-forming bacteria.

These results suggest that the dust carry microorganisms into the atmosphere, facilitate the dispersal of biological particles and might play a significant role in downwind ecosystem and human health.

The studies on transportation change of dustborne microorganisms from dust source region of China to Korea and Japan, and the seasonal variation of the microorganisms are now in progress.

Acknowledgements

This research was supported by the Global Environment Research Fund (RF-072) provided by the Ministry of the Environment, Japan. We thank Dr. Akira Taketo for his suggestion on this study.

Staff members of Meteorological Bureau of Dunhuang City gave us kind technical supports during the balloon-borne observation.

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Fig.1
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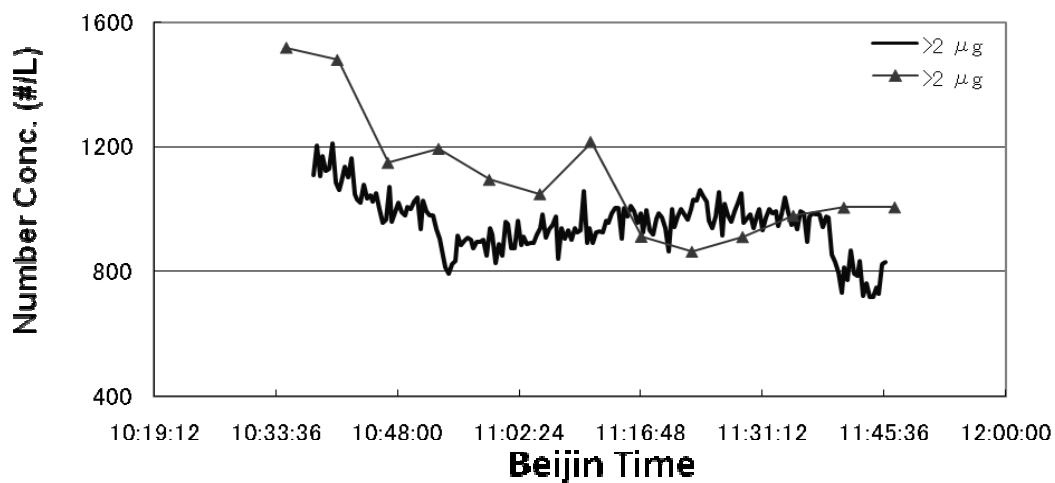
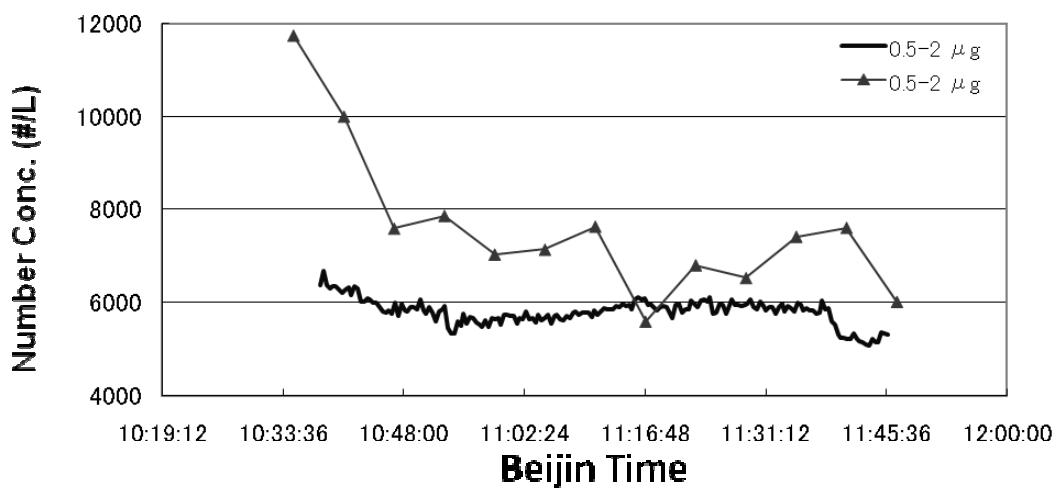
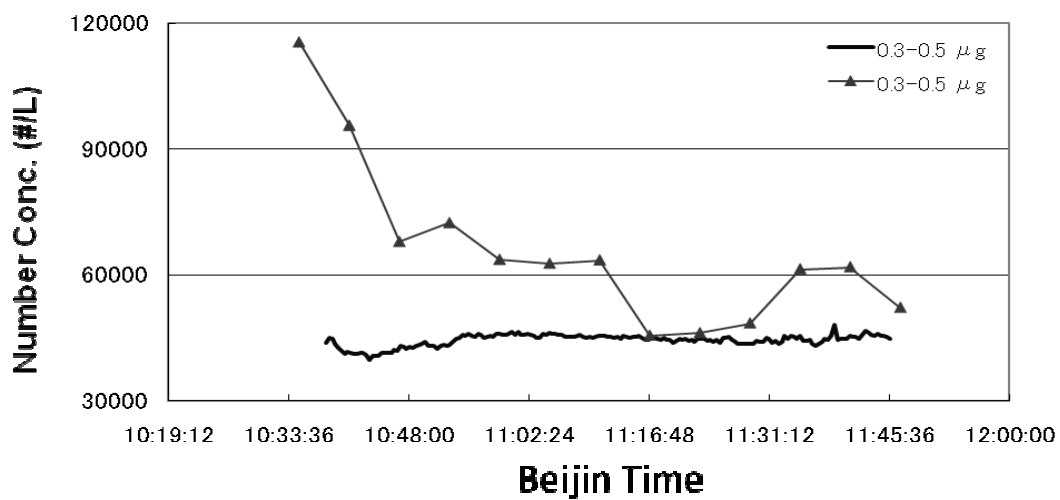


Fig.2
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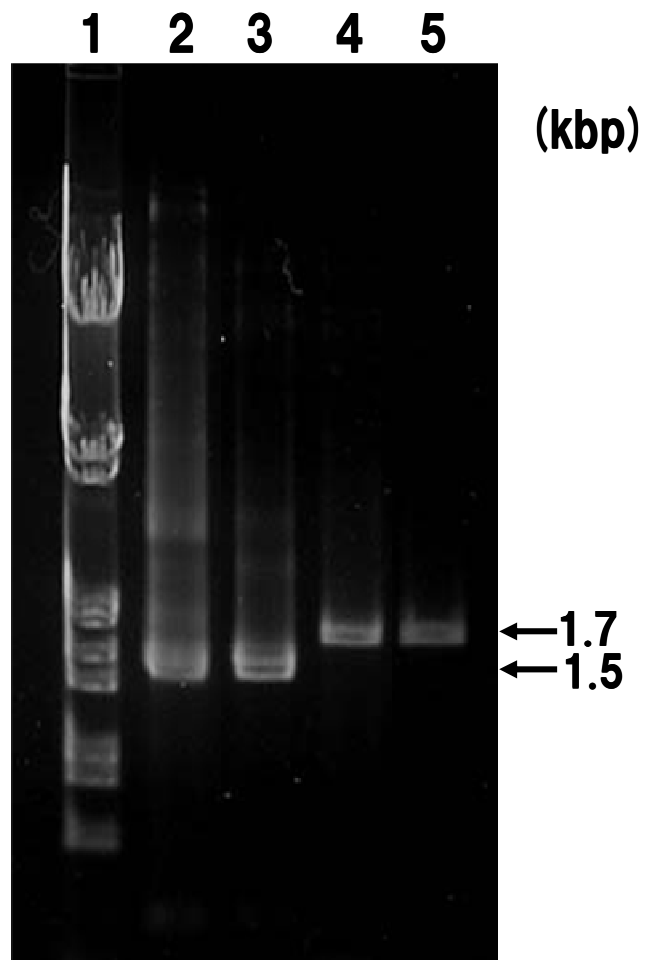


Figure Legends

Figure 1. Particle concentrations in the atmosphere of the heights of 800 m (--) and 10 m (▲) at Dunhuang, China (on 17 August 2007).

Figure 2. Amplification of rDNA of microorganisms from dusts sampled in Dunhuang, Gansu Province, China. 16S rDNA (lane 2 from 10 m height dusts, and lane 3 from 800 m dusts) and 18S rDNA (lane 4 from 10 m dusts and lane 5 from 800 m dusts) were run together with size marker (lane 1) in 1% agarose gel.