Short Communication

Soil Microbial Community Structure in an Asian Dust Source Region (Loess Plateau)

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The bacterial community structure in four geographically isolated arid regions on the Loess plateau, China, a source of Asian dust, was investigated using a 16S rRNA gene. Denaturing gradient gel electrophoresis and sequencing demonstrated that community diversity in the Loess plateau was low, and a common *Alphaproteobacteria* phylotype was identified. Phylogenetic analyses of arid soils revealed that most phylotypes had low similarity with known strains in various phyla, suggesting that these regions contain phylogenetically divergent and unknown bacteria.

Key words: arid region, community structure, diversity, Asian dust, rRNA gene

The atmospheric transport of desert soil across oceans has been well documented (2, 17, 26). Hundreds of millions of tons of dust are dispersed in the Northern Hemisphere each year (8). The majority of soil dust in the atmosphere originates from two locations: the Sahara and Sahel regions of Africa, and arid regions in China and Mongolia (13, 30). Dust storms originating in Asia usually occur annually between February and May. Since arid soil contains at least 10^5 bacterial cells g^{-1} (23, 31), large amounts of bacteria can be transported long distances in conjunction with the soil particles. The global dispersal of dust-borne microorganisms may play an important role in the transborder movement of microorganisms and thereby influence regional microbial ecology and human health in downwind environments (15, 33).

In order to elucidate the dynamics of dust-borne microorganisms, an understanding of the soil microbial community structure and its geographical variability as well as its abundance in the dust source region is essential. Current scientific understanding of microbial biogeography is particularly weak for the bacteria of arid soils (11). Much of the recent work in soil microbial ecology has focused on cataloging the diversity of soil bacteria and specific environmental changes or factors influencing bacterial communities; however, the number of microbial biogeography studies is small (20). Some biogeographic studies on an intercontinental scale have reported that specific species may be significantly differentiated by distance but did not correlate these findings with other environmental variables (6, 24, 27). Other studies on soil bacterial communities using PCR-based techniques, have shown that bacterial diversity and composition are largely independent of geographic distance or latitude on an intercontinental scale (11).

The aim of the present study is to understand the geographical variability of bacterial communities in Asian dust

source regions on a distance scale ranging from km to hundreds of km and elucidate the community structures. With the advent of ribosomal RNA gene analyses that permit the characterization of bacterial communities without culturing, it is now possible to describe the biogeographical patterns exhibited by microorganisms on large spatial scales. A genetic fingerprinting technique, denaturing gradient gel electrophoresis (DGGE), permits the simultaneous analysis of multiple samples although caveats and problems associated with the approach exist. Many studies have been performed in order to define changes in bacterial communities associated with seasonal or environmental perturbations, and spatial variability (1, 4, 9, 22).

The Taklamakan and Gobi deserts, and the Loess plateau in China are major sources of atmospheric dust in Asia. Extending over four provinces, the Loess plateau covers some 600,000 km² of northeast China and is home to more than 60 million people. Most of the region suffers from desertification, erosion and deforestation. In this study, we used DGGE fingerprinting and sequencing of the 16S ribosomal RNA gene amplified with universal bacterial primer sets to characterize the bacterial community structures and genetic diversity on the Loess plateau.

Soil samples were collected from four geographically distant regions (northwest of Yulin [Yulin NW], southeast of Yulin [Yulin SE], Taiyuan, and Huangling) of the Loess plateau in 2004 and 2005 during the winter season when the frequency of dust events is low (Fig. 1). Sampling zones were 100 to 450 km apart. Loess is composed of highly erosion-prone soil that is susceptible to the forces of wind and water. Annual precipitation on the Loess plateau is 200–650 mm. There is great seasonal variation in precipitation and most rainfall occurs between May and September.

Fifty-gram samples of surface soil (5 cm depth and 30 cm diameter) were collected in duplicate from 10 sites at Yulin NW (4 to 53 km apart), 6 sites at Yulin SE (6 to 57 km apart), 8 sites in Taiyuan (6 to 36 km apart), and 4 sites in Huangling (4 to 8 km apart). Physicochemical properties of

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Fig. 1. Sampling sites on the Loess plateau of China. (A) Large-scale map of four geographically distant regions. (B) Sampling sites around Yulin. (C) Sampling sites in Taiyuan.

the samples are shown in Table 1. The general soil texture is sand at Yulin NW (98% of $>50\ \mu\text{m}$ of particle size), sandy loam (48% of $>50\ \mu\text{m}$, 39% of $10\text{--}50\ \mu\text{m}$, 13% of $<10\ \mu\text{m}$ of particle size) at Yulin SE, and medium loam at Taiyuan and Huangling (11% of $>50\ \mu\text{m}$, 50% of $10\text{--}50\ \mu\text{m}$, 39% of $<10\ \mu\text{m}$ of particle size) (18). For measurements of pH and total organic carbon (TOC), one gram of soil sample was suspended in 9 mL of deionized water, and shaken for 30 min at room temperature. The supernatant was subjected to measurements. The TOC concentration was determined using a TOC analyzer (TOC-5000, Shimadzu, Kyoto, Japan).

DNA was extracted from the soils using an Ultraclean Soil DNA Isolation kit (Mobio Laboratories, Solana Beach, CA, USA) and the modified procedure described in the instruction manual. One gram of soil sample was mixed with 2-mL bead solution tubes, and blended with a Mini-bead-beater (Biospec Products, Bartlesville, OK, USA) for 90 s. The suspensions were subsequently frozen in liquid nitrogen and then thawed at room temperature three times in succession. Next, DNA was extracted as described in the instruction

manual. The DNA was further purified with a Wizard DNA clean up kit (Promega, Madison, WI, USA). 16S rRNA gene fragments were amplified using the primers EUB933-GC-clamp and EUBr1387 (16), which are specific for universally conserved bacterial 16S rRNA gene sequences including the V6, V7, and V8 regions. PCR amplification was performed as described by Iwamoto *et al.* (16).

DGGE was run as described by Iwamoto *et al.* (16). Approximately 300 ng of PCR product was loaded onto a 6.5% (w/v) polyacrylamide gel cast in $1\times\text{TAE}$ (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA, pH 8.0). The polyacrylamide gels (acrylamide:bisacrylamide, 37.5:1) were made with denaturing gradients ranging from 50 to 70%. DGGE gels were scanned with a Fluorimager (Molecular Dynamics Japan, Tokyo, Japan) for digitalization using a 488 nm argon laser. Images were analyzed by Image Quant version 4-2-J (Molecular Dynamics Japan) to generate a densitometric profile. To distinguish from the background, bands were detected when the relative peak area to total peak area exceeded 0.5%. Separate DGGE gels were run loaded with

Table 1. Descriptions of samples collected

Geographical region	Site	pH (H ₂ O)	TOC (mg L ⁻¹)	<i>S</i>	<i>H</i>
Northwest of Yulin	Ynw2	8.3	18	14	0.65
	Ynw3	8.3	16	14	1.00
	Ynw7	8.0	23	11	0.88
	Ynw9	8.0	18	13	1.0
	Ynw10	8.0	16	15	1.1
	Ynw11	8.0	17	12	0.94
	Ynw12	10	170	11	1.1
	Ynw13	8.8	10	11	0.88
	Ynw14	8.6	26	8	0.80
	Ynw20	7.7	20	9	0.66
Southeast of Yulin	Yse31	8.0	30	12	0.87
	Yse36	8.4	28	14	1.0
	Yse37	8.0	45	12	1.0
	Yse39	8.0	34	12	1.1
	Yse41	8.2	12	12	1.1
	Yse47	8.0	35	14	0.71
Taiyuan	T2	7.8	36	13	1.1
	T5	8.1	13	10	0.96
	T10	7.9	11	20	1.2
	T16	8.1	12	11	0.92
	T18	8.2	32	13	1.1
	T19	7.9	21	3	0.47
	T22	8.1	13	9	0.88
	T24	8.0	16	19	1.3
Huangling	H1	8.0	22	ND	ND
	H2	7.9	36	13	0.92
	H3	8.4	47	ND	ND
	H4	8.4	6.7	ND	ND

TOC, total organic carbon; *S*, OTU richness; *H*, Shannon-Weaver diversity index; ND, not determined

samples from each region to allow for direct intra-region comparison. Comparative gels were run with selected samples from each region to allow inter-region comparison. To confirm the reproducibility of the separation of PCR-amplified 16S rRNA gene fragments, the PCR amplification and DGGE analysis were performed in duplicate with subsamples of extracted DNA.

To evaluate the richness of sample diversity, the Shannon-Weaver diversity index (*H*) (29) was used. Richness (*S*) was defined as the number of operational taxonomic units (OTU) present. The Shannon-Weaver diversity index was calculated as follows: $H = -\sum_{i=1}^n P_i (\log_{10} P_i)$, where the summation is the total number of unique bands *i*, and *P_i* is the relative abundance of band *i*. The abundance of a particular fragment can be determined by using the peak area intensity in fluorescent units. The statistical significance of differences in *S* and *H* among regions was determined with Student's *t* test.

For intra- and inter-regional comparisons of bacterial communities, DGGE banding patterns were analyzed by multi-dimensional scaling (MDS) as described by Iwamoto *et al.* (16). For this purpose, the presence or absence of bands and their intensity over all profiles were recorded in a binary matrix, which was then analyzed with SPSS 9.0 J for Windows (SPSS Japan, Tokyo, Japan). The resulting graphical representation showed every band pattern as one plot where similarity in community structure can be visualized and interpreted as the distance between sites.

To determine the common species in soil samples taken from all four regions, DGGE gel bands containing 16S rRNA gene fragments were excised with a razor blade and soaked in DNA-free water at 4°C overnight. After centrifugation at 8,000×*g* for 10 min at 4°C to remove insoluble materials, the supernatant was subjected to PCR amplification using the EUBf933-GC-clamp and EUBr1387 primers. The PCR products were purified with a Minelute PCR purification kit (Qiagen), and cloned into pGEM-T Easy Vector System (Promega) with *Escherichia coli* JM109 as the host. Sequencing and phylogenetic analyses were performed as described below.

To obtain more descriptive information about the soil microbial community, genomic libraries were constructed from four samples from Huangling (H1, H2, H3, and H4). PCR products amplified using EUBf933-GC-clamp and EUBr1387 were cloned into the pGEM-T Easy Vector System with *E. coli* JM109 as the host. Twenty to 40 clones in each site were amplified with M13 primers. The diluted PCR products were further amplified with EUBf933-GC-clamp and EUBr1387, and DGGE was performed. Clones which had a unique band were sequenced and then phylogenetically analyzed.

Selected clones were sequenced in both directions on a CEQ8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA) with M13 primers (GenBank accession numbers DQ825346 to DQ825389). Similarity searches of the sequences (227 to 489 bp) were performed with FASTA from the European Bioinformatics Institute (<http://www.ebi.ac.uk>) (25).

The diversity of microbial communities in samples from Yulin and Taiyuan is represented by indices based on DGGE fingerprinting (Table 1). OTU richness and the Shannon-Weaver index varied throughout the samples. Despite the geographical separation of sampling sites at Yulin and Taiyuan, there were no significant differences in the means for OTU richness or diversity indices (*p*<0.05). Community diversity in the Loess plateau varied between 3 and 20 detectable DGGE bands per sample. These values are low in comparison to those for mesic soil communities (22). The low richness values (*S*) may be due to the extreme environmental parameters of arid soils, which are exposed to recurrent desiccation events, temperature extremes, as well as limited organic materials (9, 21, 28).

The distribution of phylotypes in each region was compared by dividing DGGE bands into categories based upon the number of samples with a shared band (Fig. 2A). A total of 42 phylotypes were observed throughout the 10 samples in Yulin NW. Among them, 18 phylotypes appeared to be unique to the sample. Samples in Yulin SE (6 sites) and Taiyuan (8 sites), had a total of 36 and 50 phylotypes, respectively. Overall, a majority of bands were sample-specific or were shared in only two samples, suggesting a high level of diversity within each region. However, one common band was observed in 9 of 10 samples from the Yulin NW, one common band was in all 6 samples from Yulin SE, and one common band was present in 6 of 8 Taiyuan samples.

After separate DGGE gels with samples from each region were run to allow for direct intra-region comparisons, representative samples were chosen for Yulin NW (Ynw2,

Fig. 2. Variability of bacterial community structures on the Loess plateau. (A) Number of OTU for each region by the number of samples in which a DGGE band is shared. (B) Graphic representation of inter-regional comparisons of the DGGE profiles among the four regions. A black column indicates the existence of a band. (C) Two-dimensional plots of MDS analysis from DGGE patterns of a subset of samples from the four regions. The stress value of the MDS plots was 0.17. H, Huangling; T, Taiyuan; Ynw, northwest of Yulin; Yse, southeast of Yulin.

Ynw12, and Ynw20), Yulin SE (Yse31, Yse39, Yse41, and Yse47), Taiyuan (T2, T16, and T22), and Huangling (H2) and the community fingerprints were compared in the same gel (Fig. 2B). In Yulin NW and Yulin SE samples, there were four and one region-specific bands, respectively. In Taiyuan and Huangling, there was no region-specific band. That is, all bands were shared by samples from Yulin NW or Yulin SE.

MDS analyses were specifically aimed at probing differences in community structure in samples from different regions on a distance scale of some hundred km (Fig. 2C). MDS comparisons of DGGE banding patterns from the four regions revealed that the variability among Yulin NW sites (*e.g.* between Ynw12 and Ynw20) was larger than the overall variability among the four regions. These results suggest that the variability of a dominant species at the 16S rRNA gene sequence level over distances of tens of km can exceed the overall variability over distances of even hundreds of km.

Despite the variability in the DGGE banding pattern, two common bands were produced with samples from the four regions used in Fig. 2B, one from 10 of 11 samples (band 1), and one from 9 of 11 samples (band 2). The band 1 nucleotide sequence (459 bp; GenBank accession number DQ825388) was identical to uncultured bacterium clone I-9 from perchloroethylene-contaminated ground water in Korea (AY625143) which belonged to the *Alphaproteobacteria*. The band 2 sequence (459 bp; DQ825389) was 99% similar to an uncultured family *Sphingomonadaceae* bacterium (*Alphaproteobacteria*) found on rice plants in Japan (AB114612). These two *Alphaproteobacteria* phylotypes are likely able to adapt to the arid environment of each Loess plateau region.

DGGE analysis allows a quick comparison of profiles from related microbial communities. An advantage of DGGE is that selected bands can be sequenced, and thus, the presence of a particular phylotype can be monitored in the environmental samples studied. However, regarding the quality of the sequences, PCR products which derive from similar sequences would have similar electrophoretic mobility (32). Co-migration of bands that are different in sequence would

decrease gel resolution, and make band identity less informative. In order to confirm single band product, several clones were analyzed after cloning of excised bands in this study. One way to improve the reliability of DGGE is to combine several molecular tools such as FISH, quantitative PCR, and clone libraries.

In the present study, genomic libraries were constructed to further characterize the bacterial community structures. The soil sample from Huangling (H2) plotted in the center of the MDS community fingerprints in Fig. 2C. In preliminary experiments, DGGE profiles showed that the variability among Huangling sites was smaller than the variability in the other three regions (data not shown). In order to obtain more descriptive information about the relatively close soil microbial communities, the samples from Huangling (H1, H2, H3, and H4) were also sequenced and phylogenetically analyzed. A total of 42 unique clones, which had a novel DGGE band, were subjected to a phylogenetic analysis. The summarized results of the FASTA search are presented in Table S1. Overall, a large array of bacterial phyla was found. *Proteobacteria* were the most abundant, followed by the *Bacteroidetes*, *Gemmatimonadetes*, *Actinobacteria*, *Cyanobacteria*, *Chloroflexi*, *Firmicutes*, *Chlamydiae*, and *Nitrospirae*. Among *Proteobacteria*, *Alphaproteobacteria* were the most abundant (Table S1). The two common phylotypes (bands 1 and 2) determined by DGGE also belonged to *Alphaproteobacteria* but identical sequences were not found in the clone libraries. Perhaps the sequenced number of clones from Huangling sites might be limited (7). Other phylogenetic studies on desert soils have shown that bacterial communities were dominated by *Acidobacteria*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* (5, 10, 19). *Proteobacteria* and *Bacteroidetes* are quite common in arid soils (3, 12).

Phylogenetic analyses of the arid soils from Huangling sites showed that only 21% of the recovered phylotypes had 97% or higher similarity with known strains (Table S1). Sixty percent of the total phylotypes (particularly phylotypes which belonged to *Gemmatimonadetes*, *Chloroflexi*, *Firmicutes*, *Chlamydiae*, and *Nitrospirae*) were distantly related to well-characterized microbial groups, suggesting that phylo-

genetically divergent and as yet unknown species attached to soil particles may be blown to geographically distant locations.

Research on dust-borne microorganisms has shown that the number of culturable bacteria and fungi in the atmosphere significantly increased when African dust occurred compared to when it did not (14). They reported that the proportion of dust-borne microorganisms was dominated by bacteria rather than fungi in samples taken in the US Virgin Islands and Bamako, and identified species belonging to the *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and others. A systematic study linking dust-borne microorganisms and soil microbial communities in dust source regions could lead to a better understanding of microbial ecology related to the global movement of dust particles.

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