THE BACTERIAL DIVERSITY IN A SOUTHERN ARIZONA PRISTINE FOREST ECOSYSTEM

Gejiao Wang 1, Derek J. Fairley2, Susheela Y. Carroll 3, Christopher Rensing 3 and Ian L. Pepper 3
(1 State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, P. R. of China; 2 Queen’s University Environmental Science & Technology Research Centre, The Queen’s University of Belfast, Belfast, BT9 5AG, UK; 3 Department of Soil, Water and Environmental Science, The University of Arizona, Tucson, AZ 85721, USA)

ABSTRACT: This study was conducted to assess the bacterial population in a southern Arizona pristine forest soil. DNA was extracted from the soil and 16S rRNA gene fragments were amplified by PCR using universal 16S rDNA primers. PCR products were ligated into the pGEM-T Easy vector and transformed into Escherichia coli DH5α to construct a 16S rDNA clone library of the soil microbes. A total of 192 clones from the library were screened for PCR-RFLP analysis and their partial 16S rDNAs were sequenced. The results indicated that the library includes 135 MseI and RsaI restriction endonuclease types and the coverage of the 16S rDNA library is 70 percent. Phylogenetic analysis suggests that the dominant bacteria in this pristine soil belong to Parachlamydiaceae, Firmicutes, Acidobacterium, a-Proteobacteria, CFB group, β-Proteobacteria, TM6 group, Bdellovibrionaceae, γ-Proteobacteria, Sorangineae, OP10 group and TM7 group.

INTRODUCTION

The structure and diversity of soil micro-organisms provide the basic microbial function of a soil ecosystem. Several surveys of microbial populations have been conducted from different pristine soils in northern Arizona using 16S rDNA direct amplification of soil DNA with universal 16S rDNA primers. A total of 21 bacterial divisions were identified, and the microbial populations among different types of soils were similar. Acidobacterium was the most dominant group, followed by Proteobacteria, Verrucomicrobiales, Bacteroides-Cytophaga-Flexibacter, Gram-positive bacteria, Green nonsulfur bacteria, Planctomycetales, Nitrospira and some new divisions (Kuske et al., 1997; Dunbar et al., 1999; Dunbar et al., 2000; Dunbar et al., 2002).

To gain knowledge of soil microbial population in southern Arizona pristine forest soil, we analyzed a typical pristine soil from the Madera Canyon in the Southern Arizona region near the border of northwestern Mexico. The canyon has an elevation of about 1,700 meters with progressive vegetation changes from desert scrub to oak woodland to pine forest as the elevation increases. To our knowledge, this study is the first of its kind to examine the natural soil microbial community in this area. In order to generate an inclusive description of the microbial community of this pristine soil, we used 16S rDNA analysis to characterize the soil microbial populations.

MATERIALS AND METHODS

Soil collection and enumeration of total culturable bacteria. Madera soil was collected in June, 2004 from the forested sites of the Amphitheater trail at the Madera Canyon Reservation Center near Green Valley, AZ, USA. This reservation center belongs to the Coronado National Forest, USDA-Forest Service. About 30 Kg of the soil was collected from the surface horizon (0-15 cm depth) of an area of about 10 m². Madera Canyon has an elevation of 1,676 meters with a vegetation of pine trees (DeBano, 1995). The soil was identified as sandy loam and it is the typical soil of the southern Arizona forest region. The field moist soil was screened through a 2-mm pore size sieve. Moisture content was determined from 5 g of triplicate soil samples dried at 105°C for 24 hrs. Soil analysis was performed at the Water Quality Center of the University of Arizona. Following sieving, soils were frozen at -20°C until DNA extraction.
Enumeration of total culturable bacteria was performed by adding 1 g (dry wt.) of each soil (triplicates) to 9 ml extraction solution [0.2 percent Na-hexametaphosphate, 6 μM Zwittergent® 3-12 detergent (Calbiochem, La Jolla, CA, USA)] and vortexing for 10 min. For heterotrophic plate counts, the soil extraction solution was serially diluted in 0.85 percent NaCl and plated onto R2A agar (Difco, Detroit, MI, USA) containing 100 mg/L cycloheximide. All plate counts were conducted after incubation of plates at 27°C for 1 week.

Analysis of microbial population of Madera soil. The total DNA was extracted from Madera soil using the UltraClean Soil DNA Kit (Mo Bio Laboratories, Solana Beach, CA, USA). Four independent DNA isolations of randomly selected soil sites were performed according to manufacture’s recommendations. The DNAs were then pooled together and further purified with QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA). The universal 16S rDNA primers Uni-338F (5'-CTCCTACGGGAGGCAGCGAG-3') and Uni-1492R (5'-GGTTACCTTGTTACGACTT-3'), which theoretically amplify 16S rDNA genes from Archaea, Bacteria and Eukarya, were used for PCR in order to construct a 16S rDNA clone library of the Madera soil micro-organisms. PCR amplification was conducted in a volume of 50 μl containing 20 ng soil DNA, 1X PCR Buffer II [50 mM KCl, 10 mM Tris-HCl (pH 8.3)], 2.5 mM MgCl₂, 200 μM each of dNTP, 0.2 μM of each primer and 2.5 U AmpliTaq Gold™ polymerase (Perkin Elmer, Foster City, CA, USA). The PCR reaction was performed by an initial hot-start denaturation step for 10 min at 95°C followed by 30 cycles of denaturation for 40 sec at 94°C, annealing for 50 sec at 50°C, and extension for 2 min at 72°C prior to a final 5 min extension step at 72°C. The amplified fragment (~1,150 bp) was analyzed by electrophoresis in a 1.5 percent low melting agarose gel following ethidium bromide staining and purified using the Gel Extraction kit (Qiagen). The purified PCR product was ligated with pGEM-T Easy (Promega, Madison, WI, USA) and transformed into E. coli DH5α by electroporation. The transformants were subsequently grown on LB agar for 24 h with ampicillin, X-Gal and IPTG according to manufacture’s recommendations. RFLP analysis was performed by digestion of each PCR product with RsaI and MseI at 37°C for 3 hrs. The digested DNA fragments were separated on 2.5 percent Metaphor agarose gels (FMC, Rockland, ME, USA) and the digestion patterns were grouped by DNA fingerprinting profiles. Phylotype richness of the Madera 16S rDNA clones was estimated by rarefaction analysis as described by Simberloff (1978).

DNA sequencing and phylogenetic analysis. Plasmids were isolated using the miniprep plasmid isolation kit (Qiagen). Primers Uni-338F was used in sequencing reactions. DNA sequencing was performed using the ABI PRISM dye terminator reaction kit (Perkin Elmer) and analyzed in an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA, USA) at the University of Arizona’s Laboratory of Molecular Systematics and Evolution sequencing facility. Nucleotide BLASTN searches (http://www.ncbi.nlm.nih.gov/BLAST) were used to locate GenBank records with similarity to the sequences of the representative 16S rDNA clones. Sequence alignments were performed using ClustalW algorithm (http://www.ebi.ac.uk/clustalw).

Nucleotide sequence accession numbers. The partial 16S rDNA sequences of the Madera soil clones were deposited at NCBI GenBank (http://www.ncbi.nlm.nih.gov) under accession numbers AY647338-1Y647427.

RESULTS AND DISCUSSION

Soil characterization and enumeration of culturable bacterial population. The Madera soil had a pH of 6.5, a moisture content of 4.6 percent, an organic carbon content of 2.6 percent and an organic nitrogen content of 2 percent. The texture of the soil was 76 percent sand, 20 percent silt and 41 percent clay. The organic carbon levels of the soil are relatively high for soils in the Southwest region of the United States. The saturated Na⁺ and K⁺ concentrations were 16 and 11 mg/Kg dry soil, respectively. Therefore the soil
was not considered saline. Heterotrophic plate counts showed that the Madera soil had a culturable bacterial population of $7.6 \times 10^7 \pm 2.8 \times 10^7$ CFU/g dry soil.

**Characterization of the 16S rDNA library from Madera soil.** To investigate the microbial community in soil, DNA was isolated from Madera soil and universal 16S rDNA primers were used to amplify the soil DNA to construct universal 16S rDNA libraries. Approximately 600 clones were generated from the library. 192 randomly selected Madera clones were analyzed both by PCR-RFLP and DNA sequencing. For a sampling size of 192 clones, the Madera library had 135 different MseI and Rsal restriction endonuclease types. The coverage of the 16S rDNA library was calculated to be 70% (135/192). A phylotype richness curve of the Madera 16S rDNA clones using rarefaction analysis is shown in Fig. 1.

**The microbial populations of Madera soil.** Sequencing analysis of the 135 polymorphic clones revealed 135 16S rDNA phylotypes. Based on the construction of phylogenetic trees, the clones were assigned into phylogenetic 15 phylogenetic Clades (data not shown). The prokaryotes of the 15 Clades were identified as Parachlamydiaceae (20 percent), Firmicutes (16 percent), Acidobacterium (13 percent), α-Proteobacteria (12 percent), CFB group (9 percent), β-Proteobacteria (8 percent), TM6 group (7 percent), Actinobacterium (4 percent), Verrucomicrobiales (4 percent), Cystobacterineae (2 percent) and 1 percent each of Bdellovibrionaceae, γ-Proteobacteria, Sorangineae, OP10 group and TM7 group (Fig. 2). Only bacterial lineages were identified from the Madera soil using the universal 16S rDNA primers. No Archaea, and Eukarya were identified in this study.

![Fig. 1. Phylotype richness of the Madera and Oversite soil bacterial 16S rDNA clones using rarefaction analysis. A total of 192 16S rDNA clones were analyzed and 135 of them were polymorphic RFLPs.](image-url)
CONCLUSION

The microbial populations of the Madera soil showed some degree of overlaps with those of northern Arizona soils. For example, Acidobacterium, Proteobacterium, Verrucomicrobiales, TM6 and TM7 groups were both found in both the northern Arizona soils and the Madera soil. The microbial diversity level of the Madera soil was also similar to those of the northern Arizona soils. An interesting cluster of Madera clones is the Parachlamydiaceae (Chlamydiales) group that constitute up to 20 percent of the total microbial population. Species of Parachlamydiaceae are recognized as intracellular endosymbionts in protozoa (Fritsche et al. 2000) and have been found in an Australian soil before (Liesack and Stackebrandt, 1992). The occurrence of Parachlamydiaceae in Madera soil suggests that such potentially pathogenic Parachlamydiaceae may be widespread in pristine soils. In this study, only bacterial lineages were identified from the Madera soil using the universal 16S rDNA primers. However, we recently also identified Archaea (Crenarchaeota division) from the Madera soil using PCR with archaeal-16S rDNA specific primers (Fan et al., 2006). Our emphasis on examining microbial diversity of such pristine soil provides valuable information of the microbial population in the Southern Arizona region and the data is useful for further investigation of the biological roles of these micro-organisms.

ACKNOWLEDGEMENTS

We thank the Coronado National Forest, USDA-Forest Service for granting us permission to collect Madera Canyon soil. We also thank Karen Josephson for soil sampling and Terry Gentry for his valuable critique. Above works were supported by NSF grant 421860 to CR and by NIEHS Basic Superfund grant 5 P42 ESO4940-09.

REFERENCES


