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Soil bacterial community composition across different topographic sites characterized by 16S rRNA gene clones in the Fushan Forest of Taiwan

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ABSTRACT. Bacterial communities present in soils from the valley, middle-slope, and ridge sites of the Fushan forest in Taiwan were characterized using 16S rDNA analysis of genomic DNA after polymerase chain reaction amplification, cloning, and denaturing gradient gel electrophoresis analysis. Phylogenetic analysis revealed that the clones from nine clone libraries included members of the phyla Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Gemmatimonadetes, Nitrospirae, Planctomycetes, candidate division TM7, and Verrucomicrobia. Members of Proteobacteria, Acidobacteria, and Actinobacteria constituted 49.1%, 32.3%, and 6.3% of the clone libraries, respectively, while the remaining bacterial divisions each comprised less than 6%. The ridge site exhibited the most bacterial species number, indicating the influence of topography. Bacterial composition was more diverse in the organic layer than in the deeper horizons. In addition, bacterial species numbers varied across the gradient horizons.

Keywords: 16S rDNA library; Acidobacteria; Bacterial community; DGGE; Proteobacteria; Topography.

INTRODUCTION

Soil microbes are essential components of the biotic community in natural forests and are largely responsible for ecosystem functioning (Hackl et al., 2004). The microbial composition of the soil surface horizon has been far better studied than that of the deeper horizons (Agnelli et al., 2004). Microbes in the deeper horizons also play an important role in ecosystem biogeochemistry (Madsen, 1995). It is not clear whether the subsurface microbial community is closely related to the surface microbial community or is an independent ecosystem with a distinct assemblage of microorganisms (Fierer et al., 2003).

About 1% of the total number of microbes present in soil is culturable (Schoenborn et al., 2004), hindering analysis of microbial diversity using culture-based methods. Various biochemical and molecular techniques have been used to more completely and precisely characterize microbes from the natural environment (Liu et al., 2006). Although every method has its advantages and limitations, 16S rRNA gene-based molecular techniques have commonly been used to analyze the phylogenetic diversity of bacterial communities (Chow et al., 2002). Polymerase chain reaction (PCR) amplification of 16S rDNA followed by separation of the PCR products on a denaturing gradient gel electrophoresis (DGGE) is an important method for analysis of bacterial communities (Muyzer et al., 1993). Bacterial species can be identified by generation of 16S rDNA clone libraries followed by sequencing and comparison with databases of ribosomal sequences, enabling phylogenetic affiliation to cultured and uncultured microorganisms (Maidak et al., 1999). These techniques have proven very suitable for comparative fingerprinting of soil samples (Watanabe et al., 2004).

A number of studies have shown that even small-scale topographical landforms can alter environmental conditions, which in turn retard or accelerate the activity of organisms (Scowcroft et al., 2000). The effects of topographical landforms on species composition, productivity, environmental conditions, and soil characteristics have been well investigated (Barnes et al., 1998), but very few studies have investigated the effects of these different environmental conditions on microbial diversity.

The Fushan forest is one of the four natural forest sites in the Taiwan Long Term Ecological Research Network (TERN) to study the effect of environmental disturbances such as typhoon and acidic deposition on ecosystem function (Lin et al., 2000; Lin et al., 2003b; King et al., 2003; Liu et al., 2004). However, a few studies have been

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conducted with Fushan forest soils: N mineralization and nitrification rates (Owen et al., 2003), fluvial transportation and sedimentation (Jen et al., 2006), and microbial diversity (Tsai et al., 2007). In the present study, clone libraries of 16S rDNA amplified fragments were used to analyze the composition of bacterial communities from three topographic sites in the Fushan forest. In addition, soil characteristics, environmental conditions, and bacterial diversity were compared in order to investigate topographic effects on bacterial diversity.

MATERIALS AND METHODS

Site description

Fushan forest, located in northern Taiwan (24°34' N, 121°34' E), has an elevation ranging between 400 m and 1,400 m and is a moist, subtropical, mixed evergreen/ hardwood broad-leaf forest with a flora of over 500 species. Plant species belonging to the families Lauraceae, Fagaceae, and Theaceae are dominant in this forest. Mean annual precipitation is approximately 3,990 mm (Owen et al., 2003). According to the Keys of Soil Taxonomy, the soils of Fushan forest belong to Hapludults, Dystrochrepts, Udipsamments, and Udorthents (Lin et al., 1996). Soil samples were collected from three locations; the valley, middle-slope, and ridge, which differed from each other in altitude, slope, characteristic plant species, and chemical characteristics (Table 1). The ridge site has the highest floral diversity and density (Lin et al., 2003a). The diversity gradually decreases through the middle-slope and valley habitats. The ridge site also has the greatest elevation, effective soil depth, and mean canopy height. The middle-slope area has the highest slope, and the valley has the greatest canopy gaps and the shallowest soil formation, due to erosion.

Soil sampling

During September 2005, soil samples were collected from the organic layer (above the topsoil, thickness approximately 5-10 cm), topsoil (depth 0-20 cm), and subsoil (depth 21-40 cm) in the valley, middle-slope, and ridge. Three soil cores in each site were randomly collected and separated into different soil layers. The samples were sieved to 2 mm and each soil core was analyzed separately for moisture content, pH, total organic carbon (TOC), and total nitrogen (TN). Moisture content, pH, and DNA extraction were done within 24 h of receiving the samples; other properties were analyzed within two weeks of sampling. Until the completion of all the analyses, the soils were stored at 4°C. For DNA extraction, the same layers in different cores of a site were pooled to yield a composite sample, which was extracted at least thrice and pooled for construction of the clone library. Air and soil temperatures were measured with a thermometer directly on site and under the soil at a depth of 5 cm, respectively. Characteristics of the three tested sites and different layers are shown in Table 1.

Chemical analyses

Moisture content was determined by drying the sample at 105°C overnight to a constant weight. pH was measured in 1:5 of soil: water extracts. Total organic carbon (TOC) was determined using a modified Walkey-Black method, as described by Nelson and Sommers (1982). Total nitrogen (TN) was measured using a modified Kjeldahl method (Yang et al., 1991). Chemical analyses were carried out in triplicate, and the mean values and standard deviation were expressed on a dry weight basis.

DNA extraction and purification

Genomic DNA of the soil samples was extracted from 2 g of fresh soil following a modified protocol of Krsek and Wellington (1999) with Crombach buffer (33 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) containing lysozyme (5 mg ml^{-1}) and sodium dodecyl sulfate (1%). After centrifugation, supernatants were subjected to potassium acetate and polyethylene glycol precipitation, phenol/ chloroform/iso-amylalcohol purification, isopropanol precipitation, and spermine-HCl precipitation. The crude DNA was purified using a Gene-Spin™ 1-4-3 DNA Extraction Kit (Protech, Protech Technology Enterprise Ltd, Taiwan) according to the manufacturer' s recommendations and stored at -20°C. DNA extractions were repeated to obtain at least three measurements in a composite sample.

PCR amplification of 16S rDNA

Bacterial 16S rDNA was amplified by PCR using the universal eubacterial primers 10f (5′- AGTTTGATCCTGGCTCAG-3′) and 1507r (5′-TACCTTGTTACGACTTCA CCCCA-3′). The *Escherichia coli* numbering positions (in the 16S rDNA) of the primers 10f and 1507r are 10-27 and 1507-1485, respectively (Heyndrickx et al., 1996). The 50 µl reaction contained 25 pmol of each primer, 200 µM of each dNTP (Protech), 1× PCR buffer (Protech, with $MgCl_2$), 1.5 U of Pro *T*aq DNA polymerase (Protech), and 1 µl of DNA.

PCR was performed using an Applied Biosystems 2720 Thermal Cycler (Foster City, CA, USA) with the following reaction conditions: 94°C for 5 min, followed by 35 cycles at 95°C for 1 min, 55°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products (5 µl) were examined by electrophoresis on a 1× TAE agarose gel (2% w v^{-1}) with a 100 bp DNA ladder (Promega, Madison, WI, USA) as a marker to confirm the size and approximate quantity of the generated amplicons.

Construction and analysis of clone libraries

The PCR products of the 16S rRNA genes were completely loaded onto a 2% low melting agarose gel (Invitrogen, San Diego, CA, USA). The band, with an expected size of approximately 1,500 bp, was cut and purified with a Gel Extraction Kit (Qiagen, CA, USA) following the manufacturer's instructions and subsequently

Table 1. Some environmental characteristics and soil properties of the study sites in the Fushan forest.

Variables	Valley	Middle-slope	Ridge
Altitude (m)	700	850	1000
Characteristic plant species	*Aralia bipinnata*, *Callicarpa dichotoma*, *Cyclobalanopsis gilva*, *Villebrunea pedunculata*	*Cinnamomum micranthum*	*Ilex goshiensis*, *I. uraiensis*, *Itea parviflora*, *Myrsine sequinii*, *Rhododendron ellipticum*, *Syzygium buzifolium*, *Ternstroemia gymnanthera*
Soil texture	Lithosols, stony loam	Colluviums, stony loam	Yellow soils, stony loam
Air temperature (°C)	25.6±0.6^{a}	24.8±0.6^{ab}	24.1±0.7^{b}
Soil temperature (°C)	22.5±0.6^{a}	22.5±0.6^{a}	22.2±0.7^{a}
pH			
Organic layer	4.4±0.1^{a,B}	4.3±0.1^{a,B}	4.3±0.1^{a,B}
Topsoil (0-20 cm)	4.7±0.1^{a,A}	4.6±0.1^{a,A}	4.6±0.1^{a,A}
Subsoil (21-40 cm)	4.8±0.1^{a,A}	4.7±0.2^{a,A}	4.7±0.1^{a,A}
Moisture content (g kg^{-1})			
Organic layer	531.3±5.9^{b,A}	589.2±53.1^{ab,A}	614.4±26.2^{a,A}
Topsoil (0-20 cm)	453.4±51.5^{a,B}	495.2±32.4^{a,B}	520.8±2.3^{a,B}
Subsoil (21-40 cm)	347.4±12.3^{b,C}	364.0±50.1^{b,C}	497.8±8.1^{a,B}
Total organic carbon (g kg^{-1})			
Organic layer	120.2±12.2^{b,A}	156.2±12.5^{a,A}	162.8±5.1^{a,A}
Topsoil (0-20 cm)	68.7±2.6^{c,B}	94.4±1.6^{b,B}	107.0±6.2^{a,B}
Subsoil (21-40 cm)	25.0±4.5^{b,C}	56.5±3.5^{a,C}	59.3±4.7^{a,C}
Total nitrogen (g kg^{-1})			
Organic layer	4.8±0.5^{b,A}	7.0±0.5^{a,A}	7.7±0.2^{a,A}
Topsoil (0-20 cm)	3.0±0.1^{b,B}	4.8±0.5^{a,B}	5.4±0.4^{a,B}
Subsoil (21-40 cm)	1.1±0.1^{b,C}	3.3±0.3^{a,C}	3.5±0.6^{a,C}
C/N ratio			
Organic layer	25.3±3.6^{a,A}	22.6±3.3^{a,A}	21.3±0.7^{a,A}
Topsoil (0-20 cm)	22.9±1.2^{a,A}	19.5±2.3^{b,AB}	19.8±0.4^{b,A}
Subsoil (21-40 cm)	22.7±2.9^{a,A}	17.1±1.2^{b,B}	16.9±1.6^{b,B}

Means±S.D (n = 3). Means in the same row that do not share the same lower case alphabetic superscript are significantly different at the 5% level according to Duncan's multiple range test (DMRT). Means for a variable in the same column that do not share the same upper case alphabetic superscript are significantly different at the 5% level according to DMRT. Air and soil temperature data correspond to a single date and measured at the time of sampling.

ligated into pGEM-T Vector Systems (Promega). The ligation product was transformed into competent *E. coli* JM109 cells, and the clones were isolated by blue-white screening with isopropyl-β-D-thiogalactopyranoside (IPTG, 0.2 mM) and 5-bromo-4-chloro-3-indolyl-β-D-galacto-pyranoside (X-Gal, 0.1 mM). White colonies were plated on LB agar containing 100 μg ml^{-1} ampicillin. Each plate contained approximately 100 clones, and 40 clones were randomly selected to represent each of the nine composite forest soil samples.

PCR screening of clone libraries, DGGE, and sequencing

PCR screening of 360 transformants was carried out as described by Schabereitner-Gurtner et al. (2001). The vector-specific forward primer T7 (5′-TAA TAC GAC TCA CTA TAG GG-3′) and reverse primer SP6 (5′-ATT TAG GTG ACA CTA TAG AAT AC-3′) were used in 25 μl reaction mixture containing 2.5 μl DNA extract as a template. Three hundred and fifty positive transformants were confirmed based on a length of approximately 1,500

bp. The PCR products were again amplified using the primer set GC clamp-968f (5′-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TA-3′) and 1401r (5′-GCG TGT GTA CAA GAC CC-3′) as nested PCR (Felske et al., 1997). Reaction mixtures were the same as those described for the 16S rDNA amplification. The PCR reaction conditions were: 94°C for 90 s, followed by 33 cycles at 95°C for 20 s, 56°C for 30 s, 72°C for 45 s, and a final extension step at 72°C for 10 min. PCR products, 5 µl, were analyzed by 2% (w v^{-1}) agarose gel electrophoresis. The PCR products (20 µl) were separated at 60°C on a vertical denaturing gradient gel using the Dcode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Polyacrylamide (6%) gels with gradients of 45-65% denaturants (where 100% denaturants contained 7 M urea and 40% formamide) were prepared in accordance with Muyzer et al. (1996), and a running time of 6 h at 150 V was selected as these conditions optimally separated the maximal number of bands. After electrophoresis, gels were stained with ethidium bromide (0.5 µg ml^{-1}) and photographed under UV light. The inserts of clones showing different positions in the DGGE were subsequently sequenced (Mission Biotech., Taiwan) using the primers SP6 and T7.

Statistical analysis and sequence and phylogenetic analyses

Differences in the physico-chemical variables due to site and soil depth were tested by the analysis of variance and Duncan's multiple range test with SPSS version 11.5 software. Significance was accepted at p level < 0.05.

The close relatives and phylogenetic affiliation of the sequences obtained were checked using the Basic Local Alignment Search Tools (BLAST) search program at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). Homology search (GenBank/EMBL/DDBJ) was performed using the BLAST program (Altschul et al., 1990) in the reference database. The sequences were also submitted to the Chimera Check program of Ribosomal Database Program (RDP) to identify chimeric artifacts. In order to obtain the rarefaction curves for the three sampling sites, species richness was plotted using BioDiversity Pro version 2, as instructed by the software manual (http://www.sams.ac.uk/dml/projects/benthic/bdpro/downloads.htm). To determine species diversity, the number of species was plotted against the number of individuals, a steeper curve indicating more diverse communities in the sample. This plot is robust against sample size effects, enabling comparisons to be made across sampling sites (Simberloff, 1972).

Nucleotide accession number

The 16S rDNA sequence data for the clones reported in this article have been submitted to the NCBI nucleotide sequence database under the accession numbers DQ451440-DQ451528.

RESULTS

Soil properties and environmental conditions

The physico-chemical characteristics of the soil in the valley, middle-slope, and ridge sites are shown in Table 1. The Fushan forest soil is stony loam, and the soil texture is lithosol in the valley, colluvium in the middle-slope, and yellow soil in the ridge. The soils were acidic (pH 4.3-4.8), and the pH gradually increased through the deeper layers; there were significant pH differences between the organic layer and topsoil or subsoil (p<0.05). The valley samples had the highest pH, but the pH differences among the sites were not significant (p>0.05). The TOC and TN contents of the soils were significantly higher (p<0.05) in the middle-slope and the ridge than the valley. The soil moisture content was the significantly highest in the ridge (p<0.05) among the three tested sites; while the TOC, TN, and C/N ratio were the significantly highest (p<0.05) in the organic layer among the three tested depths.

Analyses of clone libraries

Of the 350 clones analyzed by DGGE, 89 unique sequences were identified in the nine clone libraries (Table 2). Clones were members of 11 bacterial phyla: Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Gemmatimonadetes, Nitrospirae, Planctomycetes, candidate division TM7, and Verrucomicrobia. Members of Proteobacteria (49.1%) and Acidobacteria (32.3%) dominated the clone libraries. Within the phylum Proteobacteria, α- and γ-Proteobacteria were the most numerous (16.6% and 15.1%, respectively), followed by β- and δ-Proteobacteria. Actinobacteria constituted 6.3% of the clone library, and each of the remaining bacterial divisions constituted < 6% of the clone library (Figures 1 and 2).

Differences in bacterial composition across sampling sites

Rarefaction curves for the three sampling sites are

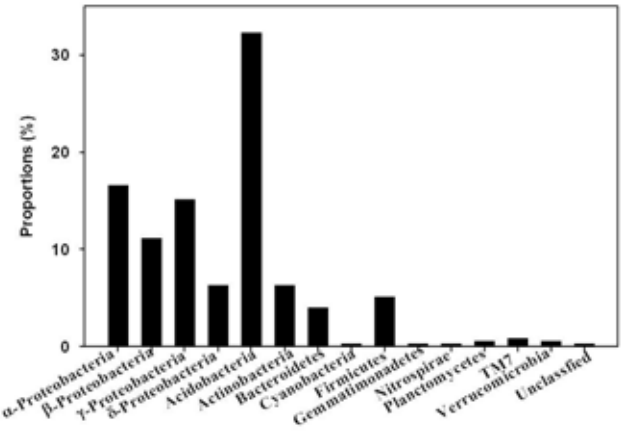

Figure 1. Phylogenetic affiliation of 16S rDNA clones (n = 350) from three soil sampling sites.

Table 2. Identification of bacterial strains in 16S rDNA clone library isolated from soils of Fushan forest.

Clone	Closest related organism in the database	Accession number of reference strain	Identity (%)	Taxon
FAC1	Acidobacteria bacterium Ellin7137	AY673303	96 (1387/1438)	Acidobacteria
FAC2	Acidobacteria bacterium Ellin7184	AY673350	96 (1410/1456)	Acidobacteria
FAC3	Uncultured Acidobacteria bacterium clone JG36-GS-146	AJ582043	97 (1412/1454)	Acidobacteria
FAC4	Uncultured Acidobacteria bacterium clone AKYH694	AY922148	96 (952/986)	Acidobacteria
FAC5	Uncultured Acidobacteria bacterium clone EB1071	AY395390	95 (1173/1224)	Acidobacteria
FAC6	Uncultured Acidobacteria bacterium clone EB1129	AY395448	98 (1369/1390)	Acidobacteria
FAC7	Uncultured Acidobacteria bacterium clone JG36-GS-146	AJ582043	96 (1400/1453)	Acidobacteria
FAC8	Uncultured *Acidobacterium* UA3	AF200699	98 (1441/1458)	Acidobacteria
FAC9	Uncultured *Acidobacterium* UA3	AF200699	97 (1428/1458)	Acidobacteria
FAC10	Uncultured *Holophaga* sp. clone JG37-AG-54	AJ519374	97 (1225/1256)	Acidobacteria
FAC46	Uncultured *Holophaga* sp. clone JG30a-KF-86	AJ536874	94 (1207/1279)	Acidobacteria
FAC52	Uncultured *Holophaga* sp. clone JG37-AG-31	AJ519368	99 (1320/1333)	Acidobacteria
FAC53	Uncultured Acidobacteria bacterium clone AKYG469	AY922023	96 (1359/1405)	Acidobacteria
FAC57	Uncultured Acidobacteria bacterium clone VHS-B3-48	DQ394942	94 (1415/1494)	Acidobacteria
FAC59	Uncultured Acidobacteria bacterium clone EB1071	AY395390	96 (1359/1407)	Acidobacteria
FAC60	Uncultured Acidobacteria bacterium clone FTL227	AF529104	94 (1325/1403)	Acidobacteria
FAC62	Uncultured Acidobacteria bacterium G03_WMSP2	DQ450698	95 (1122/1174)	Acidobacteria
FAC65	Uncultured Acidobacteria bacterium F04_WMSP1	DQ450716	95 (1299/1363)	Acidobacteria
FAC66	Uncultured Acidobacteria bacterium JG36-GS-126	AJ582044	94 (1376/1453)	Acidobacteria
FAC70	Uncultured Acidobacteria bacterium clone B08_WMSP1	DQ450707	93 (1261/1423)	Acidobacteria
FAC71	Uncultured *Holophaga* sp. clone JG30-KF-C37	AJ536864	98 (1370/1394)	Acidobacteria
FAC72	Uncultured soil baterium DUNssu164	AY913371	97 (1433/1466)	Acidobacteria
FAC77	Uncultured Acidobacteria bacterium clone C10_WMSP1	DQ450710	98 (1332/1356)	Acidobacteria
FAC80	Uncultured *Holophaga* sp. clone JG37-AG-61	AJ519377	94 (1202/1278)	Acidobacteria
FAC86	Bacterium Ellin5017	AY234434	97 (1354/1386)	Acidobacteria
FAC88	Uncultured Acidobacteria bacterium DOK_NOFERT_clone590	DQ829504	91 (479/522)	Acidobacteria
FAC11	Bacterium Ellin332	AF498714	99 (1396/1405)	α-Proteobacteria
FAC12	Bacterium Ellin6089	AY234741	96 (1394/1441)	α-Proteobacteria
FAC13	*Methylocapsa acidiphila*	AJ278726	96 (1360/1415)	α-Proteobacteria
FAC14	*Ochrobactrum* sp. 'Relman 1999'	AF028733	88 (823/928)	α-Proteobacteria
FAC15	*Stella vacuolata* DSM5901	AJ535711	92 (1323/1434)	α-Proteobacteria
FAC16	Uncultured Alpha-proteobacterium JG37-AG-26	AJ518768	96 (1218/1265)	α-Proteobacteria
FAC17	Uncultured bacterium FukuS110	AJ289986	93 (1348/1443)	α-Proteobacteria
FAC49	Uncultured Alphaproteobacterium F12_WMSP1	DQ450764	93 (1265/1355)	α-Proteobacteria
FAC58	*Acidosphaera rubrifaciens*	D86512	94 (1372/1450)	α-Proteobacteria
FAC67	Uncultured Alphaproteobacterium JG30-KF-C3	AJ536857	92 (843/908)	α-Proteobacteria
FAC68	Uncultured type II methanotroph clone 18	AY163571	95 (1307/1364)	α-Proteobacteria
FAC69	Uncultured Alphaproteobacterium clone D05_WMSP1	DQ450762	96 (905/940)	α-Proteobacteria
FAC73	Uncultured Alphaproteobacterium clone EB1033	AY395352	93 (1284/1378)	α-Proteobacteria
FAC82	Uncultured Alphaproteobacterium clone EB1127	AY395446	97 (1355/1393)	α-Proteobacteria
FAC83	*Alphaproteobacterium Shinshu-th1*	AB121772	96 (1397/1445)	α-Proteobacteria
FAC18	*Acidovorax* sp. KSP2	AB076843	97 (1445/1485)	β-Proteobacteria
FAC19	*Burkholderia* sp. TNFYE-5	AF508806	97 (1446/1489)	β-Proteobacteria
FAC20	*Delftia* sp. LFJ11-1	DQ140182	99 (1487/1490)	β-Proteobacteria
FAC21	Uncultured bacterium MS8	AF232922	96 (997/1032)	β-Proteobacteria

Table 2. (Continued)

Clone	Closest related organism in the database	Accession number of reference strain	Identity (%)	Taxon
FAC22	Uncultured Green Bay *Ferromanganous micronodule* bacterium MNC9	AF293007	96 (1438/1490)	β-Proteobacteria
FAC43	*Variovorax* sp. KS2D-23	AB196432	96 (1432/1491)	β-Proteobacteria
FAC44	Uncultured bacterium MS8	AF232922	95 (1357/1418)	β-Proteobacteria
FAC45	Uncultured bacterium clone B44	AF407722	96 (1369/1418)	β-Proteobacteria
FAC51	*Burkholderia* sp. isolate N3P2	U37344	96 (1442/1487)	β-Proteobacteria
FAC61	Uncultured Betaproteobacterium clone F03_Pitesti	DQ378169	97 (1444/1487)	β-Proteobacteria
FAC64	Uncultured Betaproteobacterium clone F03_Pitesti	DQ378169	96 (1439/1487)	β-Proteobacteria
FAC23	*Dyella japonica* XD53	AB110498	99 (1468/1481)	γ-Proteobacteria
FAC24	*Legionella*-like amoebal pathogen HT99	AY741401	96 (1426/1485)	γ-Proteobacteria
FAC25	*Pantoea agglomerans* ChDC YP1	AY691543	98 (1481/1496)	γ-Proteobacteria
FAC26	*Pseudomonas* sp. NZ096	AY014817	99 (1391/1403)	γ-Proteobacteria
FAC27	Uncultured Alteromonadales bacterium clone BL011B19	AY806128	92 (682/704)	γ-Proteobacteria
FAC28	Uncultured Gammaproteobacterium clone BIfciii1	AJ318123	92 (953/1027)	γ-Proteobacteria
FAC29	Uncultured Gammaproteobacterium YNPRH65B	AF465652	94 (1386/1460)	γ-Proteobacteria
FAC42	*Pantoea agglomerans* WAB1927	AM184266	99 (1479/1486)	γ-Proteobacteria
FAC50	Xanthomonadaceae bacterium Ellin7015	AY673181	97 (1399/1434)	γ-Proteobacteria
FAC63	Uncultured Gammaproteobacterium 308	AB252888	91 (1296/1409)	γ-Proteobacteria
FAC76	*Dyella koreensis* strain BB4	AY884571	98 (1231/1253)	γ- Proteobacteria
FAC81	Uncultured *Xanthomonas* sp. clone TM17_46	DQ279336	91 (1377/1497)	γ- Proteobacteria
FAC41	Uncultured Deltaproteobacterium clone EB1076	AY395395	93 (1376/1474)	δ-Proteobacteria
FAC48	Uncultured Deltaproteobacterium clone BSR2LA02	AY690092	90 (750/828)	δ-Proteobacteria
FAC55	Uncultured Deltaproteobacterium clone BPM3_B01	AY689889	96 (786/816)	δ-Proteobacteria
FAC56	Uncultured Deltaproteobacterium clone AKYH1423	AY921676	95 (1341/1397)	δ-Proteobacteria
FAC79	Uncultured *Entotheonella* sp. clone Dd-Ent-69	AY897120	92 (949/1031)	δ-Proteobacteria
FAC87	Uncultured Deltaproteobacterium clone KY221	AB116509	92 (904/975)	δ-Proteobacteria
FAC30	Uncultured actinobacterium Elev_16S_853	EF019692	99 (1351/1361)	Actinobacteria
FAC31	Uncultured actinobacterium Amb_16S_1709	EF019097	98 (1349/1363)	Actinobacteria
FAC74	Uncultured Actinobacterium clone CrystalBog1D10	AY792234	94 (1317/1389)	Actinobacteria
FAC75	Uncultured Actinobacterium clone CrystalBog1C4	AY792233	94 (1310/1393)	Actinobacteria
FAC78	*Frankia* sp. symbiont in root nodule FE37	AF063641	92 (1348/1457)	Actinobacteria
FAC32	Uncultured Bacteroidetes bacterium clone BIti15	AJ318185	94 (878/931)	Bacteroidetes
FAC33	Uncultured Bacteroidetes bacterium clone SW30	AJ575720	95 (1402/1470)	Bacteroidetes
FAC54	Uncultured *Flexibacter* sp. clone TM19_36	DQ279370	91 (1158/1265)	Bacteroidetes
FAC34	*Cylindrospermum* sp. PCC 7417	AJ133163	92 (1320/1433)	Cyanobacteria
FAC35	Bacillus weihenstephanensis	AB021199	99 (1495/1504)	Firmicutes
FAC36	*Veillonella parvula* strain ATCC 17745	AY995769	99 (1490/1494)	Firmicutes
FAC40	Bacillaceae bacterium KVD-1700-08	DQ490381	99 (1470/1477)	Firmicutes
FAC37	Uncultured Gemmatimonadetes bacterium clone EB1081	AY395400	98 (985/995)	Gemmatimonadetes
FAC38	Uncultured Green Bay *Ferromanganous micronodule* bacterium MNC2	AF293010	96 (1444/1495)	Nitrospirae
FAC39	Uncultured bacterium SBR2013	AF269000	91 (1221/1329)	TM7
FAC47	*Isophaera* sp.	X81958	95 (1209/1272)	Planctomycetes
FAC84	Uncultured Verrucomicrobia subdivision 3 bacterium clone EB1106	AY395425	95 (1400/1463)	Verrucomicrobia
FAC89	Verrucomicrobia bacterium clone B-E3	DQ516404	97 (539/555)	Verrucomicrobia
FAC85	Uncultured soil bacterium clone C062	AF507696	96 (1361/1405)	Unclassified

shown in Figure 3. The ridge site exhibited a more diverse microbial composition than the middle-slope or valley site. Cluster analysis (Figure 4) showed that the middle-slope and ridge sites clustered together (similarity) with a Jaccard index (*Ji*) of 22.7%, and the valley site formed another cluster sharing 19.4% *Ji* with the other two sites. Among different soil layers, topsoil and subsoil cluster together, leaving the organic layer as a separate cluster in the middle-slope and ridge sites. However, in the valley, the organic layer and topsoil clustered together. Proteobacteria, Acidobacteria, Actinobacteria, and Firmicutes were evenly distributed across the three sites, as indicated by the exact number of clones. At all three sampling sites, Proteobacteria were dominant. Gemmatimonadetes and Nitrospirae were only observed in the organic layer of the valley. Planctomycetes and Verrucomicrobia were found only in the organic layer of the middle-slope and ridge while Cyanobacteria were

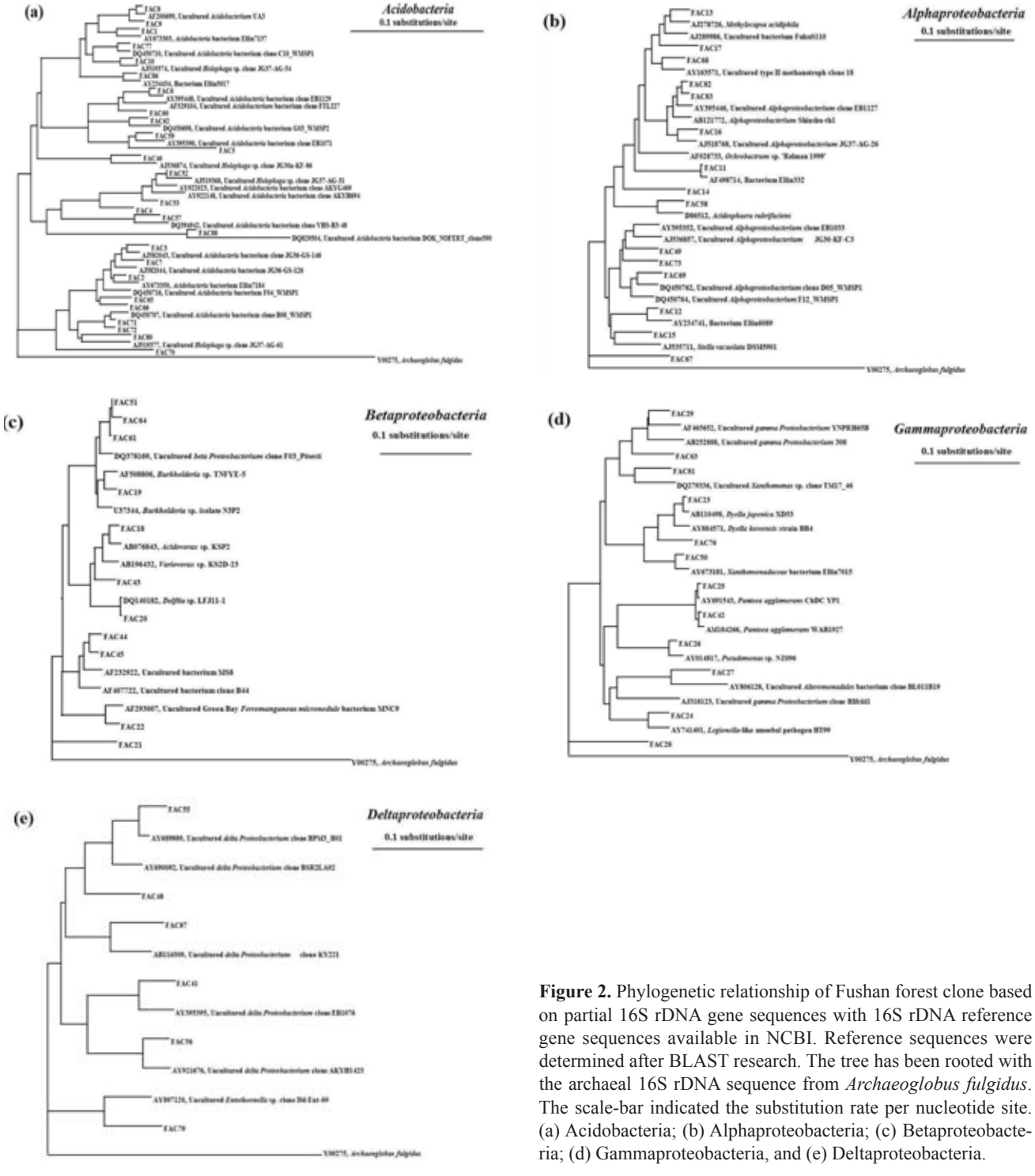

Figure 2. Phylogenetic relationship of Fushan forest clone based on partial 16S rDNA gene sequences with 16S rDNA reference gene sequences available in NCBI. Reference sequences were determined after BLAST research. The tree has been rooted with the archaeal 16S rDNA sequence from *Archaeoglobus fulgidus*. The scale-bar indicated the substitution rate per nucleotide site. (a) Acidobacteria; (b) Alphaproteobacteria; (c) Betaproteobacteria; (d) Gammaproteobacteria, and (e) Deltaproteobacteria.

found in the organic layer of ridge. The candidate division TM7 was only found in the subsoil of the middle-slope (Table 3).

Differences in bacterial composition across soil depths

The relative abundance of specific microbial groups at the three soil depths are shown in Table 3. The bacterial composition was the most diverse in the organic layer. The reduction in number of bacterial species with progressive depth was greater at the ridge site than in the middle-slope or valley site. For the ridge site, topsoil and subsoil exhibited 25.0% and 40.6% reductions in the total species number when compared to the organic layer. The reductions in number of bacterial species in the valley of topsoil and subsoil were 14.8% and 33.3%, respectively, and the middle-slope area had values of 16% and 24%. The proportional abundance of Gram-positive bacteria (Acidobacteria and Actinobacteria) increased with soil depth while the proportional abundance of Gram-negative bacteria (Proteobacteria) decreased with soil depth.

DISCUSSION

Soil at Fushan forest was acidic (pH 4.3-4.8), and the pH gradually increased through the deeper layers, as has been reported for the spruce, hemlock and grassland soils of Tatachia forest in Taiwan (Yang et al., 2003, 2006; Cho et al., 2008). Of the three sampling sites, the valley samples had the highest pH due to the lowest amount of organic matter, which was a result of large canopy gaps and consequently low leaf littering. However, the differences were not significant (p>0.05). The TOC and TN contents of the soil were the highest in the ridge, followed by the middle-slope, and those in the valley were the lowest, which correlates with the floral density. Lin et al. (2003a) reported that the biomass of woody debris was higher in the ridge (36.1 Mg ha^{-1}) than in the valley (8.5 Mg ha^{-1}).

Acidobacteria and Proteobacteria are generally the most numerically dominant phyla in soil while members of Bacteroidetes and Firmicutes are less common (Dunbar et al., 1999; Chow et al., 2002; Fierer et al., 2005). In this study, we also found Proteobacteria and Acidobacteria to be dominant. Members of α-Proteobacteria were also found to be the most abundant in the 16S rDNA clone libraries derived from Long-Term Soil Productivity (LTSP) forest soil from British Columbia, Canada (Chow et al., 2002), Australian forest soils (Stackebrandt et al., 1993), Scotland grassland rhizosphere soil (McCaig et al., 1999), and fertilizer-applied soil (Toyota and Kuninaga, 2006). Members of Acidobacteria were the most abundant in the clone libraries from Arizona pinyon pine rhizosphere and bulk soils (Dunbar et al., 1999), and in desert, prairie, and forest soils (Fierer et al., 2005). The representation of phyla in this study is very similar to that reported by Kraigher et al. (2006) using a clone library from fen soil with high organic carbon content (150 g kg^{-1}). Among the Proteobacteria, α-Proteobacteria were the most prevalent, followed by β-Proteobacteria and γ-Proteobacteria (Figure 2). Similarly, the proportions of Actinobacteria, Bacteriodetes, and Firmicutes in the clone library of this study (4.6%, 2.6%, and 3.8%, respectively) were similar to those reported by Kraigher et al. (2006) (6.1%, 3.5%, and 2.6%, respectively). The Fushan soils also had high levels of TOC (25.0-162.8 g kg^{-1}), implying the influence of organic carbon in determining bacterial diversity. The relative abundance of Actinobacteria was substantially lower in the rDNA clone library than in clone libraries from mineral soil of Williams Lake LTSP plots (Axelrood et al., 2002) and Neocaledonian mine spoils (Hery et al., 2005). Krave et al. (2002) also reported very low (1.4%) representation of Acidobacteria in a clone library of litter samples from a tropical pine forest.

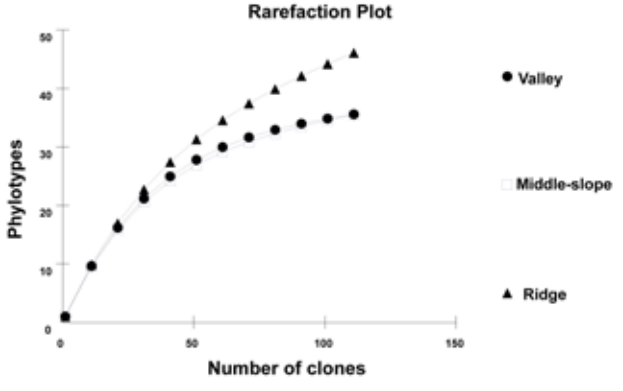

Figure 3. Rarefaction curves for the three sampling sites.

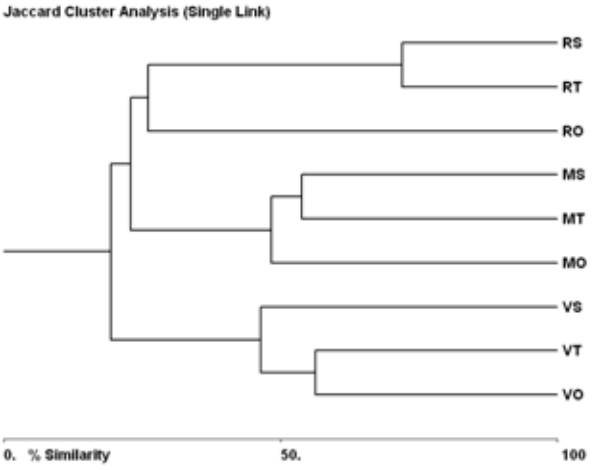

Figure 4. Dendrogram indicating the relationship among soil samples according to the identified bacterial strains from nine clone libraries. Soil sites were compared using Biodiversity Pro software. The tree was constructed using the Jaccard distance equation and single linkage method. VO: organic layer of valley; VT: topsoil of valley; VS: subsoil of valley; MO: organic layer of middle-slope; MT: topsoil of middle-slope; MS: subsoil of middle-slope; RO: organic layer of ridge; RT: topsoil of ridge; and RS: subsoil of ridge.

Table 3. Phylogenetic affiliation of bacterial 16S rDNA clones obtained from the organic layer (O), topsoil (T), and subsoil (S) of Fushan forest soil at three sampling sites.

Phylogenetic group	Number of 16S rDNA clones									Total
	Valley			Middle-slope			Ridge			
	O	T	S	O	T	S	O	T	S	
Proteobacteria, total	22	23	17	23	20	17	24	14	12	172
α-Proteobacteria	8	7	6	3	10	6	9	2	6	58
β-Proteobacteria	7	7	5	7	1	2	7	1	2	39
γ-Proteobacteria	2	5	3	8	9	9	7	7	3	53
δ-Proteobacteria	5	4	3	3	2	0	2	2	1	22
Acidobacteria	11	13	18	8	13	11	6	14	19	113
Actinobacteria	2	1	2	3	1	3	2	3	5	22
Bacteroidetes	1	2	0	0	3	3	1	3	1	14
Cyanobacteria	0	0	0	0	0	0	1	0	0	1
Firmicutes	2	1	0	4	3	1	3	2	2	18
Gemmatimonadetes	1	0	0	0	0	0	0	0	0	1
Nitrospirae	1	0	0	0	0	0	0	0	0	1
Planctomycetes	0	0	0	1	0	0	1	0	0	2
TM7 (candidate division)	0	0	0	0	0	3	0	0	0	3
Verrucomicrobia	0	0	0	1	0	0	1	0	0	2
Unclassified, total	0	0	0	0	0	0	1	0	0	1
Total clones	40	40	37	40	40	38	40	36	39	350
Total species number	27	23	18	25	21	19	32	24	19	

The higher total species number and diversity of the ridge samples relative to the valley and middle-slope samples (Table 3) might be due to the dense vegetation and floral diversity in this area, which creates specific niches for a diverse bacterial community. The bacterial community in the valley was different from that in the other two sites likely because of the high soil pH and low soil TOC, TN, and moisture content. Noguez et al. (2005) also reported that bacterial diversity was high in the hilltop and middle-slope of two tropical deciduous forests on the western coast of Mexico. Ng et al. (2006) reported that the percentages of Proteobacteria and Cyanobacteria in soils from the Taroko National Park of Taiwan were 36% and 31%, respectively. Further, Cyanobacteria percentages at high altitudes exceeded those at lower altitudes. The same phenomenon was also observed in Fushan forest soils, with the Cyanobacteria only detected in the high altitude ridge samples.

The bacterial species number was the highest in the organic layer and decreased through the topsoil and subsoil. Aeration and organic substrate supply decreased with the increasing soil depth, leading to reduction of the bacterial species number and the elimination of bacteria unable to withstand harsher conditions. The reduction of bacterial species number in the topsoil and subsoil was greater in the ridge site than in the valley or middle-slope site. Other studies using phospholipid fatty acid analysis (Blume et al., 2002), fluorescence *in situ* hybridization (Kobabe et al., 2004), and terminal restriction fragment length polymorphism analysis (LaMontagne et al., 2003) have also shown a significant reduction in the species number of soil microbial communities with changes in soil depth. Proteobacterial abundance decreased with the increasing soil depth while Acidobacteria and Actinobacteria increased with the increasing soil depth. Surface soils are rich in organic matter from the input of root exudates, surface litter, and root detritus. This energy source changes its composition and structure throughout the profile of a forest soil, from the surface to the deeper horizons (Agnelli et al., 2002). The rates of C input to the lower horizons are generally low, and the C tends to be of limited lability (Trumbore, 2000). In the same way, the water: air ratio, and the amount of oxygen available are also limiting factors. Furthermore, the surface soil has wider variations (both daily and seasonally) in temperature and moisture than soils at deeper layers (Brady and Weil, 2002). Such different conditions or gradients in resource availability and environmental stress of the pedogenetic horizons could have segregated the microbial communities. These factors are likely to be the primary factors for the vertical distribution of the different groups of bacteria such as Proteobacteria, Acidobacteria, and Actinobacteria.

The vertical distributions of Gram-negative and Gram-

positive bacteria at different soil layers are consistent with the patterns observed for other soil profiles: generally, Gram-negative dominance at the soil surface shifts to Gram-positive dominance at the deeper soil depths (Blume et al., 2002; Fierer et al., 2003). These results indicate that the bacterial communities in the deeper horizons were not simply diluted analogs of communities in the surface soils and that some microbes dominated only in the deeper soil horizons.

Torsvik et al. (1996) demonstrated that information about the bacterial communities and their diversities are needed to address the impact of environmental factors on ecosystem function. Microbial indicators are valuable to the assessment of soil quality and ecological management of the forest (Staddon et al., 1999). Future investigation might address the relationship between soil bacterial communities and forest ecosystem functions using the long-term research sites across climatic gradients in the subtropical zone in Asia.

CONCLUSIONS

In summary, the composition of bacterial communities in Fushan forest soils using a 16S rDNA clone library was documented, which could be used to construct special DNA primers and probes to target bacterial groups of interest. The clone library from Fushan forest soils represented eleven known bacterial divisions, Proteobacteria being the most dominant. The ridge soils exhibited the greatest bacterial species number, suggesting that the species number is affected by topography. However, the influence of the floral density and diversity, which creates specific niches for a variety of organisms, cannot be underestimated. Further, the increasing pH through the deeper layers also tends to influence the existence of different bacteria in different layers. Bacterial species number was the greatest in the organic layer and decreased through the deeper soil layers due to vertical gradient of nutrients, especially the form and the availability of organic substrates. Differences in the chemical composition of litter and root exudates might be expected to affect the availability of carbon sources, which would subsequently influence the prevalence of microbes.

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LITERATURE CITED

Agnelli, A., J. Ascher, G. Corti, M.T. Ceccherini, P. Nannipieri, and G. Pietramellara. 2004. Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biol. Biochem. **36:** 859-868.

Agnelli, A., L. Celi, A. Degl'Innocenti, G. Corti, and F.C. Ugolini. 2002. The changes with depth of humic and fulvic acids extracted from fine earth and rock fragments of a forest soil. Soil Sci. **167:** 524-538.

Altschul, S.F., W. Gish, M. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. **215:** 403-410.

Axelrood, P.E., M.L. Chow, C. Radomski, J.M. McDermott, and J. Davies. 2002. Molecular characterization of bacterial diversity from British Columbia forest soils subjected to disturbance. Can. J. Microbiol. **48:** 655-674.

Barnes, B.V., D.R. Zak, S.R. Denton, and S.H. Spurr (eds.). 1998. Forest Ecology. Fourth ed., Wiley, New York. 774 pp.

Blume, E., M. Bischoff, J. Reichert, T. Moorman, A. Konopka, and R. Turco. 2002. Surface and subsurface community structure and metabolic activity as a function of soil depth and season. Appl. Soil Ecol. **592:** 1-11.

Brady, N. and R. Weil. 2002. The Nature and Properties of Soils, Pearson Education, Inc., Upper Saddle River, NJ, pp. 960.

Cho, S.T., S.H. Tsai, A. Ravindran, A. Selvam, and S.S. Yang. 2008. Seasonal variation of microbial populations and biomass in Tatachia grassland soils of Taiwan. Environ. Geochem. Health. **30:** 255-272.

Chow, M.L., C.C. Radomski, J.M. McDermott, J. Davies, and P.E. Axelrood. 2002. Molecular characterization of bacterial diversity in Lodgepole pine (*Pinus contorta*) rhizosphere soils from British Columbia forest soils differing in disturbance and geographic source. FEMS Microb. Ecol. **42:** 347-357.

Dunbar, J., S. Takala, S.M. Barns, J.A. Davis, and C.R. Kuske. 1999. Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Appl. Environ. Microbiol. **65:** 1662-1669.

Felske, A., H. Rheims, A. Wolterink, E. Stackebrandt, and A.D.L. Akkermans. 1997. Ribosome analysis reveals prominent activity of an uncultured member of the class Actinobacteria in grassland soils. Microbiol. **143:** 2983-2989.

Fierer, N., J.P. Schimel, and P.A. Holden. 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. **35:** 167-176.

Fierer, N., J.A. Jackson, R. Vilgalys, and R.B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. **71:** 4117-4120.

Hackl, E., S. Zechmeister-Boltenstern, L. Bodrossy, and A. Sessitsch. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Appl. Environ. Microbiol. **70:** 5057-5065.

Hery, M., A. Herrera, T.M. Vogel, P. Normand, and E. Navarro. 2005. Effect of carbon and nitrogen input on the bacterial community structure of Neocaledonian nickel mine spoils. FEMS Microbiol. Ecol. **51:** 333-340.

Heyndrickx, M., L. Vaterin, P. Vandamme, K. Kersters, and P. De Vos. 1996. Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) patterns in bacterial phylogeny and taxonomy. J. Microbiol. Methods **26:** 247-259.

Jen, C.H., J.C. Lin, M.L. Hsu, and D.N. Petley. 2006. Fluvial transportation and sedimentation of the Fushan small experimental catchments. Quarter. Intern. **147:** 34-43.

King, H.B., C.P. Liu, Y.J. Hsia, and J.L. Hwong. 2003. Interactions of the Fushan hardwood forest ecosystem and the water chemistry of precipitation. Taiwan J. Forest Sci. **18:** 367-373.

Kobabe, S., D. Wagner, and E.M. Pfeiffer. 2004. Characterisation of microbial community composition of a Siberian tundra soil by fluorescence *in situ* hybridization. FEMS Microb. Ecol. **50:** 13-23.

Kraigher, B., B. Stres, J. Hacin, L. Ausec, I, Mahne, J.D. van Elsas, and I. Mandic-Mulec. 2006. Microbial activity and community structure in two drained fen soils in the Ljubljana Marsh. Soil Biol. Biochem. **38:** 2762-2771.

Krave, A.S., B. Lin, M. Braster, A.M. Laverman, N.M. van Straalen, W.F.M. Röling, and H.W. van Verseveld. 2002. Stratification and seasonal stability of diverse bacterial communities in a *Pinus merkusii* (pine) forest soil in central Java, Indonesia. Environ. Microbiol. **4:** 361-373.

Krsek, M. and E.M.H. Wellington. 1999. Comparison of different methods for the isolation and purification of total community DNA from soil. J. Microbiol. Methods **39:** 1-16.

LaMontagne, M.G., J.P. Schimel, and P.A. Holden. 2003. Comparison of subsurface and surface soil bacterial communities in California grassland as assessed by terminal restriction fragment length polymorphisms of PCR-amplified 16S rRNA genes. Microb. Ecol. **46:** 216-227.

Lin, K.C., C.T. Duh, F.C. Ma, and H.H. Wang. 2003a. Biomass and nutrient content of woody debris in the Fushan subtropical broadleaf forest of northeastern Taiwan. Taiwan J. Forest Sci. **8:** 235-244.

Lin, K.C., S.P. Hamburg, S. Tang, Y.J. Hsia, and T.C. Lin. 2003b. Typhoon effects on litterfall in a subtropical forest. Can. J. Forest Res. **33:** 2184-2192.

Lin, K.C., F.W. Horng, W.E. Cheng, H.C. Chiang, and U.C. Chang. 1996. Soil survey and classification of the Fushan experimental forest. Taiwan J. Forest Sci. **11:** 159-174.

Lin, N.H., C.T. Lee, C.C. Chan, W.C. Hsu, M.B. Chang, W.L. Lin, C.A. Hong, H.B. King, and Y.J. Hsia. 2000. A preliminary analysis of chemical characteristics of atmospheric pollutants and their deposition budget on the Fushan forest in Taiwan. Terrest. Atmos. Ocean. Sci. **11:** 481-500.

Liu, C.P., H.B. King, M.K. Wang, Y.J. Hsia, and J.L. Hwong. 2004. Water chemistry and temporal variation of nutrients in stemflow of three dominant tree species in the subtropics of the Fushan forest. Water Air Soil Poll. **155:** 239-249.

Liu, B.R., G.M. Jia, J. Chen, and G. Wang. 2006. A review of methods for studying microbial diversity in soils. Pedosphere **16:** 18-24.

Madsen, E. 1995. Impacts of agricultural practices on subsurface microbial ecology. Adv. Agronomy **54:** 1-67.

Maidak, B.L., J.R. Cole, C.T. Parker Jr, G.M. Garrity, N. Larsen, B. Li, T.G. Lilbum, M.J. McCaughey, G.J. Olsen, R. Overbeek, S. Pramanik, T.M. Schmidt, J.M. Tiedje, and C.R. Woese. 1999. A new version of the RDP (Ribosomal Database Project). Nucleic Acids Res. **27:** 171-173.

McCaig, A.E., L.A. Glover, and J.I. Prosser. 1999. Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl. Environ. Microbiol. **65:** 1721-1730.

Muyzer, G., E.C. De Waal, and A.G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. **59:** 695-700.

Muyzer, G., S. Hottentrager, A. Teske, and C. Wawer. 1996. Denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA: A new molecular approach to analyse the genetic diversity of mixed microbial communities. *In* A.D.L. Akkermans, J.D. van Elsas, and F.J. de Bruijn (eds.), Molecular Microbial Ecology Manual, 3.4.4, Kluwer, Dordrecht, pp. 1-27.

Nelson, D.W. and L.E. Sommers. 1982. Total carbon, organic carbon and organic matter. *In* A.L. Page (ed.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. 2nd ed. The American Society of Agronomy, Wisconsin. pp. 539-580.

Ng, C.C., W.C. Huang, C.C. Chang, W.S. Tzenga, T.W. Chen, Y.S. Liu, and Y.T. Shyu. 2006. Tufa microbial diversity revealed by 16S rRNA cloning in Taroko National Park, Taiwan. Soil Biol. Biochem. **38:** 342-348.

Noguez, A.M., H.T. Arita, A.E. Escalante, L.J. Forney, F. García-Oliva, and V. Souza. 2005. Microbial macroecology: highly structured prokaryotic soil assemblages in a tropical deciduous forest. Global Ecol. Biogeogr. **14:** 241-248.

Owen, J.S., M.K. Wang, C.H. Wang, H.B. King, and H.L. Sun. 2003. Net N mineralization and nitrification rates in a forested ecosystem in northeastern Taiwan. Forest Ecol. Manage. **176:** 519-530.

Schabereitner-Gurtner, C., G. Pinar, W. Lubitz, and S. Rölleke. 2001. An advanced molecular strategy to identify bacterial communities on art objects. J. Microbiol. Methods **45:** 77-87.

Schoenborn, L., P.S. Yates, B.E. Grinton, P. Hugenholtz, and P.H. Janssen. 2004. Liquid serial dilution is inferior to solid media for isolation of cultures representative of the phylum-level diversity of soil bacteria. Appl. Environ. Microbiol. **70:** 4363-4366.

Scowcroft, P.G., D.R. Turner, and P.M. Vitousek. 2000. Decomposition of Metrosideros polymorpha leaf litter along elevational gradients in Hawaii. Global Change Biol. **6:** 73-85.

Simberloff, D. 1972. Properties of the rarefaction diversity

measurement. Amer. Naturalist. **106:** 414-418.

Stackebrandt, E., W. Liesack, and B.M. Goebel. 1993. Bacterial diversity in a soil sample from a subtropical Australian environment as determined by 16S rDNA analysis. Fed. Amer. Soc. Exper. Biol. **7:** 232-236.

Staddon, W.J., L.C. Duchesne, and J.T. Trevors. 1999. The role of microbial indicators of soil quality in ecological forest management. Fores. Chron. **75:** 81-86.

Torsvik, V., R. Sorheim, and J. Goksoyr. 1996. Total bacterial diversity in soil and sediment communities - a review. J. Indust. Microbiol. **17:** 170-178.

Toyota, K. and S. Kuninaga. 2006. Comparison of soil microbial community between soils amended with or without farmyard manure. Appl. Soil Ecol. **33:** 39-48.

Trumbore, S. 2000. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecol. Appl. **10:** 399-411.

Tsai, S.H., A. Selvam, and S.S. Yang. 2007. Microbial community of topographical gradient profiles in Fushan forest soils of Taiwan. Ecol. Res. **22:** 814-824.

Watanabe, T., S. Aasakawa, A. Nakamura, K. Nagaoka, and M. Kimura. 2004. DGGE method for analyzing 16S rDNA of methanogenic archaeal community in paddy field soil. FEMS Microbiol. Lett. **232:** 153-163.

Yang, S.S., H.L. Chang, C.B. Wei, and H.C. Lin. 1991. Reduce waste production with modified Kjeldahl method for nitrogen measurement. J. Biomass Energy Soc. China **10:** 147-155.

Yang, S.S., H.Y. Fan, C.K. Yang, and I.C. Lin. 2003. Microbial population of spruce soil in Tatachia mountain of Taiwan. Chemospere **52:** 1489-1498.

Yang, S.S., S.H. Tsai, H.Y. Fan, C.K. Yang, W.L. Huang, and S.T. Cho. 2006. Microbial population of hemlock soil in Tatachia Mountain of Taiwan. J. Microbiol. Immun. Infect. **39:** 195-205.

利用 16S 核糖體基因選殖株分析台灣福山森林不同地形位置土壤細菌組成

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本研究經由聚合酵素鏈鎖放大反應、選殖及變性梯度凝膠電泳分析基因體 DNA 中的 16S rDNA，探討台灣福山森林溪谷、中坡及稜線土壤細菌組成。演化分析顯示九個基因選殖庫中的所有選殖株可分為 Proteobacteria、Acidobacteria, Actinobacteria、Bacteroidetes、Cyanobacteria、Firmicutes、Gemmatimonadetes、Nitrospirae、Planctomycetes、candidate division TM7 和 Verrucomicrobia 等族群。其中 Proteobacteria、Acidobacteria 和 Actinobacteria 分別佔全部選殖基因庫的 49.1%、32.3% 和 6.3%，而其他的族群則皆未超過 6%。稜線地區有最多的細菌種類，顯示地形影響。有機質層比下層土壤細菌組成多樣化。細菌種類數量亦隨不同土層而改變。

關鍵詞：16S rDNA 選殖基因庫 ；Acidobacteria；細菌組成 ； 變性梯度凝膠電泳 ；Proteobacteria；地形。