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## Bacterial phylogenetic diversity and a novel candidate division of two humid region, sandy surface soils

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### Abstract

The extent of microbial community diversity in two similar sandy surface soils from Virginia and Delaware (USA) was analysed with a culture-independent small subunit ribosomal RNA (SSU rRNA) gene-based cloning approach with about 400–700 SSU rDNA clones obtained from each sample. While there were no operational taxonomic units (OTUs) having more than three individuals, about 96–99% of the OTUs had only a single individual. The clones showing less than 85% similarity to the sequences in the current databases were fully sequenced. The majority of the clones (55%) had sequences that were more than 20% different from those in the current databases. About 37% of the clones differed by 15–20% in sequence from the database, 16% of the clones differed by 10–15%, and 5% of the clones differed by only 1–10%. Phylogenetic analysis indicated that these sequences fell into 10 of the 35–40 known phylogenetic divisions. Many of the clones were affiliated with *Acidobacterium* (35%). While a substantial portion of the clones belong to alpha (24%) and beta (12%) *Proteobacteria*, a few of them were affiliated with delta (6%) and gamma (3%) *Proteobacteria*. About 6% of the clones belong to *Planctomycetes*, and 4% of the clones were related to gram-positive bacteria. About 4% of clones were related to other bacterial divisions, including *Cytophaga*, Green sulfur bacteria, *Nitrospira*, OP10, and *Verrucomicrobia*. Eight sequences had no specific association with any of the known divisions or candidate divisions and were phylogenetically divided into three novel division level groups, named AD1, AD2 and AD3. Candidate division AD1 represented by six clones (4%) was found in both sites and consisted of two subdivisions. The community structures were similar between these two widely separated, sandy, oligotrophic, surface soils under grass vegetation in a temperate, humid climate but somewhat dissimilar to community structures revealed in similar studies in other types of soil habitats. Published by Elsevier Science Ltd.

**Keywords:** SSU rRNA genes; Phylogenetic diversity; Soil microbial community

### 1. Introduction

Microorganisms play an integral and often unique role in ecosystem functions yet we know little about dominant populations that presumably play vital roles in these functions, nor do we know much about how these populations differ with habitat. The greatest microbial diversity at small scales appears to reside in the soil. Soil microbial communities are among the most complex, diverse, and important assemblages in the biosphere.

Analysis of genetic diversity in soil communities by DNA renaturation suggests that there are approximately  $4-7 \times 10^3$  different genome equivalents per 30 g of soil (Torsvik et al., 1990), which, if extrapolated to species diversity, implies that there are at least  $10^3$  or even more species per g of soil.

The culture-independent, small subunit ribosomal RNA (SSU rRNA) gene-based molecular approaches have revealed astonishing diversity in soils and other environments (c.f., Barns et al., 1999; Borneman et al., 1996; Borneman and Triplett, 1997; DeLong, 1992; Dojka et al., 1998; Dunbar et al., 1999; Felske et al., 1998; Fuhrman et al., 1993; Hugenholtz et al., 1998a,b; Kuske et al., 1997; Zhou et al., 1997, 2002). Several studies have analyzed 17–124

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sequences from single soil samples, and yet remarkably, very little repetition has been observed among these sequences (c.f. Bintrim et al., 1997; Borneman and Triplett, 1997; Borneman et al., 1996; Felske et al., 1998; Ludwig et al., 1997; Zhou et al., 1997). Virtually all previous studies revealed novel groups with few or no known cultivated representatives at the division or phylum level. However, none of these studies have reached the level of diversity revealed by DNA–DNA hybridization methods, perhaps because only a limited number of SSU inserts have been obtained or screened in rDNA cloning experiments. Thus, despite recent expansion in divisions by several previous studies (Dojka et al., 1998; Hugenholz et al., 1998a,b), our understanding of the extent of microbial diversity is still incomplete. More studies on a variety of soil types and habitats are needed to obtain a comprehensive view of microbial community composition and structure in soil environments.

To better understand the extent of microbial diversity in soil, we used the SSU rRNA gene-based cloning approach to analyze microbial community structure of two oligotrophic, sandy surface soils from two widely-separated geographic locations (Delaware and Virginia) by screening all of the SSU rDNA inserts (up to 700) obtained in a single cloning experiment. Although several previous studies examined soil microbial community composition using SSU rDNA-based cloning approach (Bintrim et al., 1997; Borneman and Triplett, 1997; Borneman et al., 1996; Felske et al., 1998; Liesack and Stackebrandt, 1992; Ludwig et al., 1997; Stackebrandt et al., 1993; Ueda et al., 1995; Zhou et al., 1997), no studies have been performed with oligotrophic, sandy surface soil under grass vegetation in a temperate, humid climate. Thus, we also sought to determine whether the community structure detected by SSU rDNA cloning was similar for two soils with the same soil, climate and vegetation but from widely separated geographic areas. To our knowledge, this is the first study which examined microbial community composition in this habitat type by screening such a large number of clones. Our results indicate that phylogenetic diversity and distribution in these two soils are quite different from those in other soil types, with the majority of the clones (~95%) having 10–25% sequence divergence from the sequences in the current databases. In addition, several novel candidate divisions were discovered.

## 2. Materials and methods

### 2.1. Site description and soil sample collection

Two surface soil samples (Ab-2, D1-1) were collected from previously described sites (Zhou et al., 2002) near the Atlantic Coast of northern Virginia (Abbott's Pit) and central Delaware (Dover Air Force Base). The surface sample from Abbott's Pit was collected on October 19,

1995, and that from Dover Air Force Base was obtained on June 28, 1996. Both sites were covered with grass vegetation. The soil texture was medium to fine sand at the Virginia site and coarse sand at the Delaware site. Both soils are classified as Hapludults which indicates they developed under humid conditions and are highly weathered. Both samples were taken from a depth of 0–7 cm below the surface. The total organic carbon (TOC) was 4.51 and 4.08 mg/g at the Virginia and Delaware sites, respectively. Soil was collected using steam-cleaned Lexan core liners. Soil from the center of the core was removed by a sterile spatula, collected into sterile Whirl-pak bags and immediately frozen.

### 2.2. DNA extraction, rDNA amplification and sequencing

The bulk community DNA was directly extracted from 5 g of soil, purified and SSU rRNA genes amplified as previously described (Zhou et al., 2002). Unique SSU rDNA clones were detected by restriction fragment length polymorphism (RFLP) analysis first with *Msp* I plus *Rsa* I, and for clones not resolved with *Hha* I plus *Hae* III (Zhou et al., 2002).

Unique SSU rDNA inserts were reamplified with the vector specific primers in a 100  $\mu$ l reaction using 0.2–1  $\mu$ l of the previously amplified SSU rDNA products as template. When good PCR products could not be obtained for some clones with the vector specific primers, primers from both ends of the SSU rRNA genes (28F, 68F, 1540R or 1392R) were used. The amplified PCR products were purified using Wizard™ PCR Preps DNA purification system (Promega Corp., Madison, WI) according to the manufacturer's instructions. DNA sequences were determined with the purified PCR products as template by automated fluorescent *Taq* cycle sequencing using ABI 377 Sequencers (Applied Biosystems, Foster City, CA). Approximately 30–100 ng of the purified DNA was used for one sequencing reaction.

Representative clones were first partially sequenced with the primer, 529R, which spans *E. coli* SSU rRNA gene positions of 529–512. Nearly full sequences were obtained for the clones which were 85% or less similar to the SSU rRNA genes in current databases using nine additional primers as described previously (Zhou et al., 1995).

### 2.3. Phylogenetic analysis

Sequences with the primer 529R were edited with SeqEd program (Applied Biosystems, Foster City, CA), and the full sequences were assembled with the programs phredPhrap and Consed (University of Washington, Seattle, WA). Chimeric sequences were identified by using the CHECK\_CHIMERA program (Ludwig et al., in press), by using the branching order discrepancies in phylogenetic trees inferred independently with the 5' and 3' end sequences, and by comparing all of the sequences obtained in this study with each other. The clones whose partial sequences were

available were subjected to heteroduplex analysis with polyacrylamide gel electrophoresis as described previously (Qiu et al., 2001).

All of the sequences were preliminarily analyzed by searching the current databases using the program FASTA in Genetic Computer Group (GCG) software package (Devereaux et al., 1984). Sequences were then aligned manually to the SSU rDNA sequences of the species, which showed high similarity scores in the outputs of FASTA, in the previously aligned SSU rDNA sequence database, RDP-II, release 8.0 (Maidak et al., 2000) using the GDE multiple sequence editor program from RDP. Initial phylogenetic screening was constructed using the DNA distance program, Neighbor-Joining with Felsenstein correction, in ARB (Strunk et al., in press) based on all SSU rDNA sequences of the bacterial groups, such as alpha, beta, gamma and delta *Proteobacteria*, gram positive bacteria, and *Acidobacterium*, in RDP plus the closely related SSU rDNA sequences which were absent in RDP. Based on the initial phylogenetic results, appropriate subsets of SSU rDNA sequences were selected and subjected to final phylogenetic analysis through maximum likelihood method with the program fastDNAmI in RDP. Final phylogenetic trees were

constructed with the transition/transversion ratio of 2.0 by using jumbled orders of 10 for the addition of taxa. For the sequence comparison at division level (Fig. 1), based on the bacterial mask of Lane (1991), the following homologous nucleotide positions were used in comparative analysis: 101 to 180, 220 to 450, 480 to 837, 859 to 998, 1043 to 1126, 1147 to 1165, and 1175 to 1439. Fig. 1 was constructed based on all of the sequences of Fig. 2 of Dojka et al. (1998), which identifies 40 divisions plus the nearly full length sequences obtained in this study using the DNA distance program (neighbor-joining algorithm with Felsenstein correction), in the ARB software package. Bootstrap analyses for ~14,000 replicates were performed to provide confidence estimates for tree topologies. The novel candidate divisions from this study were assigned based on the comparison of branching lengths of the tree with all other known and recently proposed candidate divisions (Dojka et al., 1998).

#### 2.4. Nucleotide sequence accession numbers

The accession numbers of the SSU rRNA gene sequences in GenBank are AY289351–AY289492.

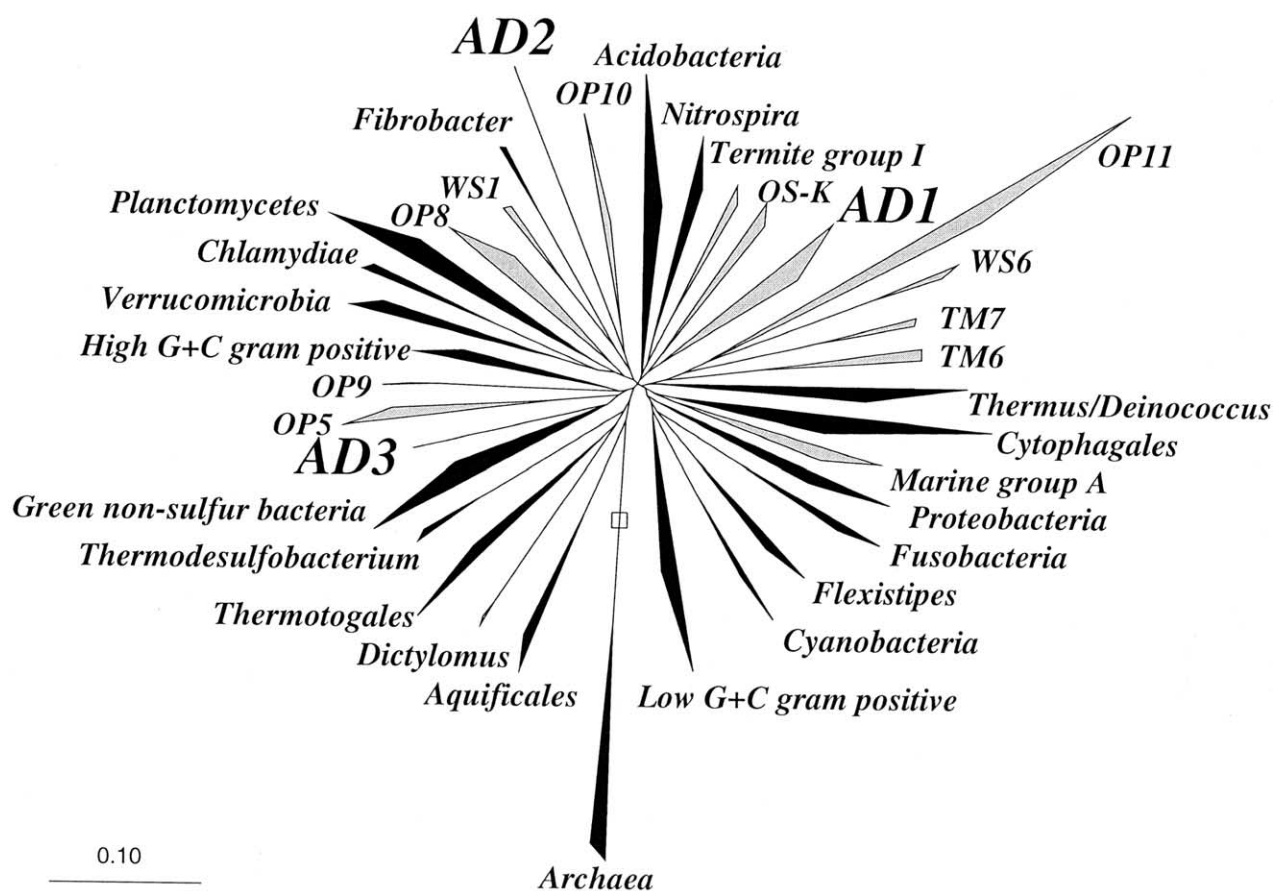


Fig. 1. Phylogenetic distance tree of the bacterial domain for all currently recognized divisions and candidate divisions. The figure is based on the divisions of Hugenholtz et al. (1998b) amended with those of Dojka et al. (1998). Novel divisions (in this study) are labeled AD. Filled wedges indicate the divisions represented by sequences found in this study. Non-filled wedges are reference divisions with no representation in these soil samples.



Fig. 2. Phylogenetic relationships of the SSU rDNA sequences present in these samples in *Acidobacterium*. The subdivisions described by Hugenholtz et al. (1998a) are represented by the numbered brackets. The “?” indicates a potentially new subdivision. This tree was established with maximum likelihood method.

### 3. Results

#### 3.1. SSU rDNA RFLP analysis

A total of 695 clones containing entire SSU rRNA inserts were obtained from the Abbott's Pit soil sample and 397 SSU rRNA clones were recovered from the Dover soil sample. A total of 627 and 339 unique restriction patterns were obtained with the first pair of restriction enzymes for the Abbott's Pit and Dover samples, respectively. Clones with unique restriction patterns are referred to as operational taxonomic units (OTUs). While there were no OTUs in the two samples having more than three individuals, about 96–99% of OTUs had only a single individual.

As reported previously, 7.4 and 10.5% of the clones were estimated to be chimeric from the 30–40 clones randomly selected from each clone library (Zhou et al., 2002). To further evaluate this important point, we evaluated the proportions of chimeric sequences based on the nearly full-length sequences in this study. Each sequence was subjected to analysis using CHECK\_CHIMERA, and by independent phylogenetic analysis based on both the 5' and 3' halves of each sequence. Since CHECK\_CHIMERA may fail to detect the chimeric molecules resulting from recombination between the parent SSU rDNA genes which are distantly related to the sequences in the current databases, the sequences were also compared to one another to increase the chance for identifying chimeric molecules (Ludwig et al., 1997). One of the 17 sequences (5.9%) from the Abbott's Pit sample and two of 23 sequences (8.7%) from the Dover sample were detected to be chimeric. The recombination sites occurred in highly conserved regions of the SSU rRNA genes such as 930–960 and around 1150. The ratios of chimeric molecules by this more detailed analysis were consistent with those of the previous screening method.

Besides PCR-generated chimeric molecules, PCR-generated heteroduplex SSU rDNA molecules could also be a problem in the SSU rRNA gene-based cloning approach (Qiu et al., 2001). Thus the clone libraries were inspected for the occurrence of heteroduplex molecules. The clones with partial sequences of the 5' end were subjected to polyacrylamide gel electrophoresis. The proportions of heteroduplex molecules were 3.6 and 4.5% for the Abbott's Pit and Dover samples, respectively. All clones determined to be chimeric or heteroduplexes were excluded from subsequent phylogenetic analyses.

#### 3.2. Phylogenetic analysis

To determine phylogenetic diversity in this habitat type, representative SSU rDNA clones of OTUs that occurred more than once in a given library, as well as representatives of some of the unique OTUs were partially sequenced. Those clones showing <85% similarity to the sequences in the current databases were fully sequenced. The majority of the clones (54.9%) had sequences that were more than 20%

different from those in the current databases. About 37% of the clones differed by 15–20% in sequence from the database, 16% of the clones differed by 10–15%, and 5% of the clones differed by only 1–10%, suggesting that the sequences of these bacterial clones were highly diverse.

The phylogenetic distribution of the Abbott's Pit and Dover soil clones was established with boot-strap Neighbor-Joining method with the sequences from all known and candidate divisions. These sequences fell into 10 of 40 known main phylogenetic divisions (Fig. 1). While a substantial portion of the clones were affiliated with *Acidobacterium* (35.2%), many of the clones (45%) fell into *Proteobacteria*. Within *Proteobacteria*, the majority of the clones belonged to alpha *Proteobacteria* (23.9%), and a few of them were beta (12.0%), delta (6.3%), and gamma *Proteobacteria* (2.8%). About 6% of the clones belonged to *Plantomycetes*, and 4% of the clones were related to gram-positive bacteria. About 4% of the clones were related to other bacterial divisions, including *Cytophaga*, Green-sulfur bacteria, *Nitrospira*, OP10, *Verrucomicrobia*. Eight of the 142 sequences had no specific association with any of the known divisions or candidate divisions and were phylogenetically divided into three novel division level groups (Candidate AD1, AD2 and AD3). Candidate AD1 was represented by six clones (4.2%). Similar to the results from the study by Dojka et al. (1998), no consistent branching order of divisions in the tree shown in Fig. 1 could be established by bootstrap Neighbor-Joining analysis. However, the topology of the three novel candidate divisions in the bootstrap Neighbor-Joining tree was consistent with the tree topology established by fast-DNAml with a subset of 98 representative sequences from all of the known and candidate bacterial divisions (data not shown).

The phylogenetic distribution of the sequences between Abbott's Pit and Dover surface soil samples was similar for the dominant divisions. For example, *Acidobacterium* was the most dominant in both Abbott's Pit (29.2%) and Dover (40.3%) samples, followed by alpha *Proteobacteria* (23.1 and 24.7% for Abbott's Pit and Dover samples), beta *Proteobacteria* (9.2 and 14.3% for Abbott's Pit and Dover samples), *Plantomycetes* (7.7 and 3.9% for Abbott's Pit and Dover samples) and delta *Proteobacteria* (6.2 and 3.9% for Abbott's Pit and Dover samples). The proportion of Gram positive bacteria was low in both Abbott's Pit and Dover samples (4.6 and 3.9%). The novel candidate division, AD1, was also observed in both Abbott's Pit and Dover samples (3.1 and 5.2%). One of the major differences was the distribution of gamma *Proteobacteria*. While about 6% of the clones were related to gamma *Proteobacteria* in the Abbott's Pit samples, no clones belonging to the gamma subdivision of *Proteobacteria* were found in the Dover sample. Some of the rare clones from *Cytophaga*, Green-sulfur bacterium, OP10 and AD3, occurred in the Abbott's Pit sample but not in Dover sample, and vice versa for

the clones from *Nitrospira*, *Verrucomicrobia* and AD2. Sampling of rare clones would be expected to be patchy.

*Proteobacteria* are the most abundant group in these two soil samples. All of the clones in alpha *Proteobacteria* fell into six subgroups. The most frequently encountered groups (ASB-366, ABS-392, ABS-432, DS-10, DS-11, DS-44, DS-45, DS-57, DS-78, DS-91, and DS-165) were affiliated with *Agrobacterium* and *Bradyrhizobium* (Figure not shown). Four clones (ABS-12, ABS-183, ABS-358, and ABS-418) were close to *Zymomonas*, whereas one clone was associated with *Azospirillum* (ABS-9), environmental clones SMK 141 (DS-22) and MC77 (DS-49), respectively. The majority of the clones (ABS-80, ABS-113, ABS-458, DS-299, DS-138, ABS-503, DS-107, DS-108, and ABS-4) in the beta subdivision of *Proteobacteria* fell into the *Rubrivivax* subgroup and were closely related to *Rubrivivax gelatinosus* (Figure not shown). While 1 clone (DS-219) was related to *Zoogloea*, 3 clones (DS-16, DS-19 and DS-90) were affiliated with the anaerobic toluene-degrading bacterium, *Azoarcus toluolyticus*. Four clones (ABS-7, ABS-173, DS-59 and DS-177) were closely related to the Australia soil clone MC114 in the delta subdivision of *Proteobacteria* (Figure not shown). The clones, ABS-13, ABS-423, DS-7, and DS-59, also fell into this subdivision. They were about 85% similar to the sequences of *Desulfuromonas* group or *Myxobacteria* group. Such levels of sequence similarity indicate that there appear to be a new group of bacteria within the delta *Proteobacteria*. Although gamma *Proteobacteria* are thought to be abundant in natural environments, only four clones were found within the gamma subdivision. They are closely related to *Pseudomonas* (ABS-635), *Xanthomonas* (ABS-59), *Legionella* (ABS-567) and *Methylmicrobium* (ABS-23).

*Acidobacterium* is a recently recognized bacterial division with only three cultivated representatives; the majority of sequences that make up this division are from environmental clones. Eight monophyletic subdivisions were identified based on the available sequences (Hugenholtz et al., 1998a). The sequences of this division from these two samples were compared with other representative sequences representing the eight recognized subdivisions (Hugenholtz et al., 1998a). Phylogenetic analysis indicates that all of the soil clones from the Abbott's Pit and Dover samples fell into five of the eight subdivisions except clone ABS-21, which may represent a novel subdivision (Fig. 2). The most frequently encountered groups were affiliated with the subdivisions 6 (40% within this division) and 4 (30%) of *Acidobacterium* (Fig. 2). The clones in subdivision 6 were closely related to the soil clone, KB2426, while the clones in subdivision 4 were related to soil clones 32–11. More than 10% of the clones within this division were affiliated with the subdivisions 1 and 7. The clones in subdivision 1 were closely related to the forest soil clone Ep\_T1.185, and the clones in subdivision 7 were similar to the soil clone iii1-8. The clones in subdivision 7 were deeply branched within this division. Another group (subdivision 3) within this division

Table 1  
SSU rRNA sequence signatures for the novel candidate divisions

Position	Consensus composition	AD1	AD2	AD3
353	A	A	T	A
570	G	G	G	G
698	G	G	G	G
812	G	G	A	C
906	G	G	G	A
933	G	G	G	G
955	U	U	C	U
976	G	G	G	G
995	C	C	C	C
1109	C	C	C	C
1198	G	G	G	G
1202	U	T <sup>3</sup> /G <sup>1</sup>	U	G
1207	G	G <sup>2</sup> /C <sup>2</sup>	C	C
1208	C	C <sup>3</sup> /T <sup>1</sup>	C	C
1224	U	G <sup>3</sup> /T <sup>1</sup>	U	U
1229	A	A	A	A
1233	G	G	G	G
1234	C	C	C	C
1384	C	C	C	C

consisted of clones DS-60, DS-95 and DS-314. They were closely related to soil clone C002. The clone ABS-21 was deeply branched within this division, and appeared to form a new subdivision within *Acidobacterium* (Fig. 2).

Sequence analyses revealed that 15 of the 19 signature nucleotides examined in the sequences of the novel candidate division AD1 exactly matched the consensus signature nucleotides in bacteria, whereas there were only partial matches to the consensus signature nucleotides at the other four signature nucleotide positions (Table 1). Secondary structure analysis with the sequence of ABS-29 in AD1 indicated that the sequence formed appropriate and meaningful 16S helix-loop structures. Similarly, the sequence in the novel candidate divisions AD2 and AD3 also had 15 of 19 signature nucleotides that matched the consensus signature nucleotides in bacteria suggesting that the cloned sequences are derived from the SSU rRNA gene sequences of members of the domain Bacteria. Phylogenetic analysis revealed two subdivisions within the novel candidate division AD1 (Fig. 3). Each subdivision consisted of three clones. While there are 88–95% similarities within each subdivision, the sequences in subdivision 1 were 80–87% similar to those in subdivision 2. In addition, subdivision 1 was observed in both Abbott's Pit and Dover samples, whereas subdivision 2 was present only in Dover samples.

Another less frequently encountered group was gram-positive bacteria. Two clones (DS-61 and ABS-14) were related to the soil clone, MC47 and *Streptoalloteichus hindustanus* IFO15115, within high G + C gram positive bacteria, with the similarities of about 90% (Figure not shown), the other four clones were affiliated with low G + C gram positive bacteria. While clones DS-241 and DS-31 were related to *Clostridium butyricum*, ABS-17 was

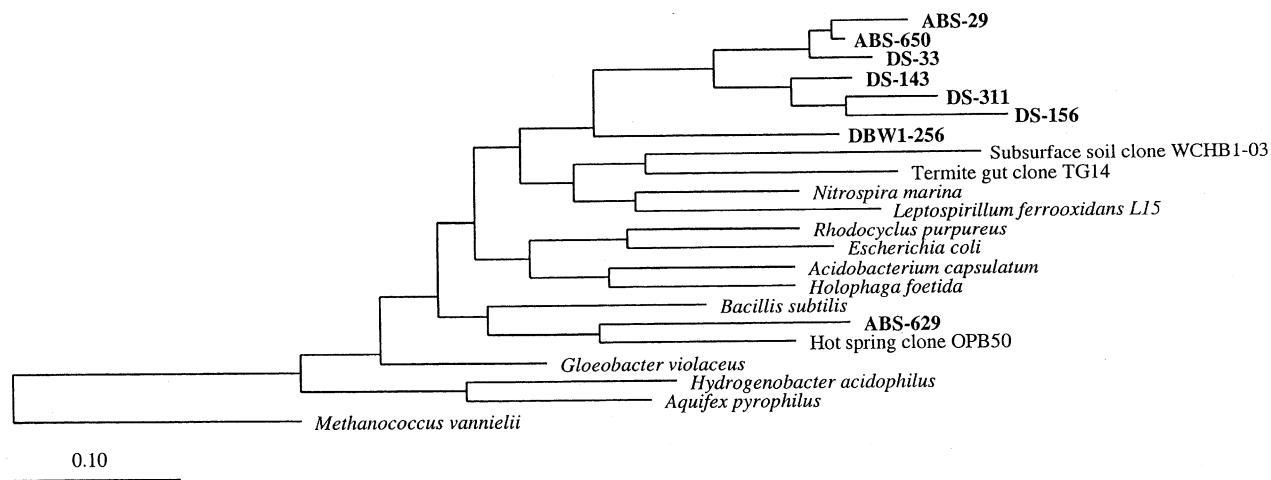


Fig. 3. Phylogenetic relationships of the SSU rDNA sequences present in these samples in the novel candidate division AD1. The sequence, DBW1-256, was obtained from subsurface soil (Zhou et al. unpublished). This tree was established with maximum likelihood method.

closely related to *Bacillus* species. Although clone ABS-138 fell into low G + C gram positive bacteria, it was only 84% similar to the closest relative, *Heliobacterium chlorum*. Such a low level of similarity could indicate a novel group of bacteria within the low G + C gram positive bacteria.

Phylogenetic analysis also showed that seven clones (ABS-2, ABS-144, ABS-157, ABS-351, ABS-368, DS-53 and DS-54) fell into the *Planctomycetes* division (Figure not shown). Four clones in this division were similar to *Pirellula staleyii*, one clone was closely related to the soil clone MC55 (91.3%). Clones DS-54 and DS-166 also fell into this division but showed distinct similarity to other known *Planctomycetes*. One clone (ABS-705) was affiliated with Green non-sulfur bacteria and showed substantial similarity to H1-43-F (83%) from deep subsurface soil (Chandler et al., 1998). In addition, while one clone was affiliated with the division of *Nitrospira*, another clone was related to the newly proposed candidate division, OPB50 (Hugenholtz et al., 1998b).

#### 4. Discussion

The majority of SSU rDNA clones obtained from the two soils in this study showed different RFLP patterns with the first restriction enzyme pair indicating that these clones appear to be substantially different at the sequence level. This is supported by the sequence-based phylogenetic analysis in this study. Although about 400–700 SSU rDNA clones from each sample were compared, no identical RFLP patterns were observed between the two surface soil samples. Also the proportion of PCR generated artifacts revealed via sequencing, especially chimeras and heteroduplexes, was at a consistent level of 5–10 and 3–5%, respectively. As with previous studies, we found that the microbial community is

highly diverse even in this oligotrophic sandy soil environment.

Sequence analysis based on SSU rRNA genes revealed the presence of possibly 13 different groups of bacteria in the two surface soils, and the majority of the clones (~95%) had 10–25% sequence divergence from the sequences in the current databases. These results suggest that these clones have a wide phylogenetic distribution, and that most are likely indicators of new genera, families, phyla or divisions. These results also imply that more studies are needed to obtain a comprehensive view of microbial community composition and structure in soil environments.

Phylogenetic analyses have resulted in the description of over 40 major lineages (divisions). While the majority of the analyzed soil rDNA sequence types were affiliated with recognized bacterial divisions, the present study suggests the existence of three novel candidate divisions (AD1, AD2 and AD3) indicating that despite recent expansion in divisions (Dojka et al., 1998; Hugenholtz et al., 1998b), our understanding of the extent of microbial diversity is still incomplete. Candidate division AD1 was represented by six sequences and found at both sites, whereas the candidate divisions AD2 and AD3 were each represented by one sequence. In addition, there are no strict criteria or rules for determining novel candidate divisions. In this study, the novel candidate divisions were assigned based on the comparisons of branching lengths of the phylogenetic tree with all other known and recently proposed divisions. It is more or less arbitrary at this stage. For example, the candidate division AD3 can be assigned as a novel division by comparing the branching lengths of Fig. 1 for all known and recently proposed candidate division, but it might also represent a deep branching member of the division, Green non-sulfur bacteria. Thus, studies of the additional sequences in the candidate divisions AD2 and AD3 are needed to verify their division level status.

Although many of the currently recognized taxonomic divisions of Bacteria are represented by the sequences from the surface soils, one sequence was also affiliated with the newly proposed candidate division from Yellowstone hot spring, OP10 (Hugenholtz et al., 1998b), suggesting that this group is not restricted to thermophilic environments. Additional sequences belonging to this candidate division have also been obtained from a hydrocarbon-contaminated subsurface soil exhibiting methanogenesis (Dojka et al., 1998). This breadth of habitat suggests that this group may be important in ecological processes and functions. Also, the association of the surface soil sequence types with the OP candidate divisions expands the known diversity for this division and strengthens its division level status.

The highly diverse bacterial division, *Acidobacterium*, appears to be widely distributed. SSU rDNA-based molecular analysis indicates that they exist in a variety of soil environments from the tropics to the tundra (Barns et al., 1999; Borneman et al., 1996; Borneman and Triplett, 1997; Dojka et al., 1998; Felske et al., 1998; Hirons et al., 1997; Hugenholtz et al., 1998a; Liesack and Stackebrandt, 1992; Ludwig et al., 1997; Nusslein and Tiedje, 1998; Stackebrandt et al., 1993; Ueda et al., 1995; Wise, 1997; Zhou et al., 1997). About 35% of the clones in these two samples were associated with five of the eight subdivisions in this division. These results further indicate that the members of *Acidobacterium* division are widely distributed in natural environments and are among the more prominent populations in soils. The broad phylogenetic diversity of the rDNA sequences in this division indicates that its members are likely to be

very diverse physiologically and ecologically. While their function and activity level is uncertain, they at least store C and bio-available N and P. However, due to their uncultivated status, little else can be said about their ecological functions.

While the phylogenetic distribution at the division level was very similar between these two sandy soils from different geographical locations, there are striking differences between the soils studied by other groups. For example, in Wisconsin agricultural soil (Borneman et al., 1996), the majority of the sequences obtained were from the *Cytophaga–Flexibacter–Bacteroides* group (21.8%), the low G + C content gram positive bacteria (21.8%) and *Proteobacteria* (16.1%). Within the *Proteobacteria*, more clones were found in the beta *Proteobacteria* subdivision (8.1%) than in alpha *Proteobacteria* (1.6%) and delta *Proteobacteria* (3.2%) subdivisions (Table 2). In contrast to Wisconsin soil, most of the phylotypes in these two soil samples were the members of *Proteobacteria* (45.0%) and *Acidobacterium* (35.2%), and only one clone (0.3%) was from *Cytophaga–Flexibacter–Bacteroides* group. Within the *Proteobacteria*, the majority of the clones in these soil samples was from alpha *Proteobacteria* (23.9%) and beta *Proteobacteria* (12.0%) subdivisions. Considerable differences for the dominant divisions were also observed between these soil samples and Amazon forest and pasture soils (Borneman and Triplett, 1997), Cosnino pinyon-woodland soils of the Arid Southwestern United States (Kuske et al., 1997), Siberian tundra soil (Zhou et al., 1995), Australian subtropical soil (Liesack and Stackebrandt, 1992; Stackebrandt et al., 1993), Japanese temperate soybean soil (Ueda et al., 1995), and peaty acid grassland

Table 2

Distribution of the bacterial divisions in soils analyzed by the SSU rRNA gene-based cloning approach. Studies with sample sizes of 25 or more 16S rDNA clones were included

Division	Abbott sandy	Dover sandy	Wis. agricul.	Arizona arid woodland	Siberian tundra	Netherlands grassland	Austral. arid	Brazil forest	Brazil pasture
<i>Acidobacterium</i>	29	40	2	51	16	19	0	18	20
<i>Proteobacteria</i>	43	43	16	10	61	19	66	12	20
α- <i>Proteobacteria</i>	23	25	2	– <sup>a</sup>	21	15	66	4	4
β- <i>Proteobacteria</i>	9	14	8	– <sup>a</sup>	9	4	0	2	0
δ- <i>Proteobacteria</i>	6	4	3	– <sup>a</sup>	26	0	0	2	8
γ- <i>Proteobacteria</i>	6	0	3	– <sup>a</sup>	5	0	0	4	8
Planctomycetes	8	4	3	2	2	0	8	16	12
Gram positive	5	4	23	7	12	59	0	22	22
<i>Cytophaga–Flexibacter–Bacteroides</i>	2	0	22	4	2	0	0	6	8
Novel/Uncertain	3	5	31	17	2	0	26	25	14
Total clones analyzed	66	76	124	168	43	27	86	49	49

Abbreviations and sources: Wis. Agricul., Wisconsin agricultural soil (Borneman et al., 1996); Arizona arid woodland soil (Dunbar et al., 1999); Siberian tundra soil (Zhou et al., 1997); Netherlands grassland soil (Felske et al., 1998); Austral. arid, Australian arid soil (Liesack and Stackebrandt, 1992); Brazil forest soil, Brazil pasture soil (Borneman and Triplett, 1997).

<sup>a</sup> Values not provided.



soils (Felske et al., 1998) (Table 2). For example, the proportion of gram positive bacteria in grassland (Felske et al., 1998) and forest soil (Borneman and Triplett, 1997) is much higher than those observed in these two surface soils. Although methodological differences and bias, such as primers used for amplification, differential lysis of species, preferential amplification of specific templates, may contribute to such differences, some of the differences are undoubtedly due to differences in climate, soil types, vegetation and land management practices. This study represents an analysis of oligotrophic, sandy soil microbial communities under grass in a temperate, humid climate. Since microbial population composition and dynamics are controlled by ecosystem conditions, it should be possible to begin to see patterns that correspond to habitat type if sample size is sufficiently large and geographical replication is adequate.

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