Phylogenetic diversity of a bacterial community determined from Siberian tundra soil DNA

Jizhong Zhou,[1]† Mary Ellen Davey,[1] Jordi B. Figueras,[1]‡ Elizaveta Rivkina,[2] David Gilichinsky[2] and James M. Tiedje[1]

Author for correspondence: James M. Tiedje. Tel: +1 517 353 9021. Fax: +1 517 353 2917. e-mail: tiedjej@pilot.msu.edu

[1] Center For Microbial Ecology, Michigan State University, East Lansing, MI 48824-1325, USA

[2] Institute of Soil Sciences and Photosynthesis, Russia Academy of Science, Pushchino, Russia

Genomic DNA was isolated from the active layer of tundra soil collected from the Kolyma lowland, Northeast Eurasia, near the Arctic Ocean coast. The SSU (small subunit) rRNA genes were amplified with eubacterial primers from the bulk genomic community DNA and cloned into plasmid vectors. Forty-three SSU rDNA clones were obtained, and all of them had different RFLP patterns. Phylogenetic analysis based on partial sequences (about 300 bp) established with the maximum likelihood method revealed the presence of three major and several minor groups that fell into 11 of the established lines of bacteria, and one sequence that could not be assigned to any of the described groups. Most of the clones belonged to the alpha (20·9%) and delta (25·6%) subdivisions of the *Proteobacteria*, with lesser proportions in the beta (9·3%) and gamma (4·7%) subdivisions, groups typically isolated from soil by culture methods. Fewer than 12% of the clones belonged to Gram-positive bacteria, and 16% of the clones were related to *Fibrobacter*. The majority of the clones (70%) had sequences that were 5–15% different from those in the current databases, and 7% of the clones had sequences that differed by more than 20% from those in the database. The results suggest that these tundra-derived clones are very diverse in phylogeny, and that many probably reflect new genera or families. Hence, most of the tundra soil bacterial community has never been isolated and thus the physiology and function of its dominant members appears to be unknown.

Keywords: small subunit rDNA, permafrost, Arctic

INTRODUCTION

Micro-organisms residing in tundra soils face conditions that are different to those experienced by temperate-soil micro-organisms, namely growth only at low temperatures, long periods in the frozen state, soil of high water content due to poor drainage sometimes causing anaerobic conditions, low pH, and high soil organic matter perhaps composed of a narrower range of microbial substrates due to more limited plant diversity in this ecosystem. These conditions may result in different taxonomic groups of micro-organisms dominating the tundra compared with the temperate-soil community. The most comprehensive evaluation of this question was done during the Tundra Biome studies of the International Biological Program. In general, summaries of that work show that heterotrophic bacteria isolated in tundra soils parallel those found in temperate soils, and no group shows any preference for the tundra environment (Dunican & Rosswall, 1974). *Pseudomonas*, *Brevibacterium* and *Arthrobacter* spp. were the dominant bacterial groups isolated in most of the tundra sites studied. Gram-negative organisms were equal in number to Gram-positives. The population density of anaerobes was between one-tenth and one-fiftieth that

Abbreviation: SSU, small subunit.

†**Present address:** Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

‡**Present address:** Institute of Aquatic Ecology, University of Girona, Girona, Spain.

The GenBank accession numbers for the sequences reported in this paper are AF016717–AF016759.

of aerobes, with isolates of *Clostridium*, sulfate reducers and methanogens most often recovered. Numbers of cellulose decomposers, actinomycetes and nitrifiers were lower than in temperate soils but this may be due to the acid conditions of many tundra peat soils.

It is well known, however, that isolated microorganisms do not reflect the true composition and structure of soil communities. Categorizing SSU (small subunit) rDNA from natural communities has become a popular alternative means to characterize communities because it avoids the limitation of culturability and directly provides information on phylogenetic affiliation. The purpose of this work was to examine the composition of a tundra soil community by the SSU rDNA approach and to compare these findings with similar information from non-tundra soils as well as with information obtained from isolate studies on tundra soils. This information can also serve as a baseline for future tundra studies and for enhancing culturing approaches.

METHODS

Site description and soil sample collection. The sampling site is located in a permafrost area on Northeast Eurasia tundra (Kolyma-Indigirka lowland): 157° E, 69° N, 7 m elevation above sea level near the mouth of Malaya Konkovaya river, 100 km from the Arctic Ocean (East Siberian Sea) coast. In this region, the summer is often warm (mean air temperature in July is +10 °C) but the winter, which has snow cover of less than 15 cm, is extremely cold (below −40 °C). The permafrost table is at 0·30 m. The seasonal thaw starts in June and reaches the maximal depth in September. The active (melted) layer is frozen for the remainder of the year. The vegetation of the site is dominated by dwarf birch (*Betula* sp.), dwarf willow (*Salix polaris*), dwarf larch (*Pinus* sp.), berries, grasses, mosses and lichens.

A melted soil sample was taken by coring from the 0·05–0·10 m depth in summer (August) from the active layer in a swamp depression. The soil is a modern peaty cryosol with a water (ice) content of 40 %. The surface of the soil core was trimmed away with a sterile knife to remove surface contamination and the remaining sample was immediately divided into segments approximately 5 cm long, placed in pre-sterilized aluminium cans, sealed and placed in frozen storage. The sample was shipped frozen on 'blue ice' to Moscow and then to Michigan State University, where it was stored at −20 °C prior to analysis. Immediately after sampling the quantity of methane (6·8 ml kg^{-1}) and the redox potential (−80 mV) were determined. The chemical composition of this sample was: ^{13}C, 27·11 %; soil organic matter 22·0 %; C(org), 11·0 %; N, 0·73 %; C/N, 15; pH (KCl), 4·7; pH (H_2O), 5·3; sulfide (H_2S), 0 %; ferrous iron, 0·012 %.

DNA extraction and SSU rRNA gene amplification. DNA was extracted from 5 g soil using a high temperature/salt/SDS-based lysis method (Zhou *et al*., 1996). One-fifth of the crude DNA (100 µl) was purified by a gel-plus-minicolumn purification method (Zhou *et al*., 1996) except that the DNA was eluted twice from the resin column. The final volume of DNA was brought to the starting volume of the aliquot.

The oligonucleotides for amplifying eubacterial SSU rRNA genes were the primer set of fD1 and rP1 as described by Weisburg *et al*. (1991) with modifications to the linker sequences (Zhou *et al*., 1995). All stocks for PCR amplification were made and procedures were performed with the precautions suggested by Kwok & Higuchi (1989). A volume of 1 µl purified DNA was used as a template in a 20 µl reaction. The conditions for amplifying SSU rRNA genes were as described previously (Zhou *et al*., 1995).

SSU rRNA gene cloning. The PCR-amplified SSU rRNA gene products were quantified by comparing the band intensity on agarose gels to the known concentrations of standard lambda DNA. The amplified PCR products were directly ligated to the pCR II vector from Invitrogen. Ligation and transformation were carried out according to the manufacturer's instructions. We used ratios of inserts to vectors in ligation of 0·5–1:1 instead of the normal 5–10:1 to help ensure a more comprehensive recovery of the more difficult to clone PCR products. A volume of 2 µl ligation reaction mixture was transformed by heat pulse into *Escherichia coli* INVαF′ competent cells (Invitrogen).

To speed up the cloning processes, a set of primers specific to the polylinker of the vector pCR II was designed. The sequences of the 5′ and 3′ end primers were: 5′GCCGCC-AGTGTGCTGGAATT3′ and 5′TAGATGCATGCTCGAG-CGGC3′. The cloned inserts were directly amplified from transformant cells using these primers. The size of the PCR-amplified product is expected to be 80 bp if no fragment is inserted into the vector. A tiny amount of cells picked up by touching the colony with a toothpick from an LB agar plate containing ampicillin was added to a standard PCR mix containing 1× *Taq* polymerase buffer, 2·5 units *Taq* polymerase (Promega), 200 µM dNTPs and 100 pmol of each primer in a 100 µl reaction. The reaction conditions consisted of denaturation at 92 °C for 2 min, 30 cycles of 94 °C for 30 s, 68 °C for 1 min and 72 °C for 2 min, plus one additional cycle with a final 6 min of chain elongation. The amplified PCR products were analysed for 16S rDNA inserts by gel electrophoresis on 1·5 % agarose gels. The specificity of the vector-targeted amplification primers was determined by Southern hybridization.

SSU rDNA RFLP analysis. The amplified PCR products of the correct size (1·5 kb) were purified by using Wizard PCR Preps DNA purification system (Promega) according to the manufacturer's instructions. To detect unique SSU rDNA clones, RFLP analysis was carried out. One-fifth of the 100 µl PCR amplified products was digested with 0·1 U restriction enzymes *Eco*RI plus *Msp*I and *Rsa*I plus *Bst*VI (Gibco-BRL Life Technologies), respectively, at 37 °C overnight. The resulting RFLP products were separated by gel electrophoresis in 3·5 % Metaphor agarose (FMC Bioproducts) in 1× TBE at 4 °C with 7 V cm^{-1} for 4 h. Metaphor agarose gel was prepared according to the manufacturer's instructions. The gel was stained with 0·5 µg ethidium bromide ml^{-1} and visualized by UV excitation. The RFLP patterns were compared by eye.

SSU rDNA sequencing. The DNA sequences of the SSU rRNA genes were determined directly using the purified PCR products as the sequencing template. DNA sequences were determined with automated fluorescent *Taq* cycle sequencing using the ABI Catalyst 800 and ABI 373A Sequencer (Applied Biosystems). Approximately 100 ng purified DNA was used for one automated fluorescent sequencing reaction. The sequencing primer (790F) for SSU rRNA gene used in this study spanned *E. coli* SSU rRNA gene position 787–802. Since no sequence information was obtained with this primer for

some clones, the vector-specific primer (TA106) was used for sequencing.

Phylogenetic analysis. Sequences were assembled using assembling programs in the Genetic Computer Group (GCG) software package (Devereux *et al*., 1984), and preliminarily analysed by searching the current databases (GenBank release 91.0 and EMBL release 44.0) using the program FASTA. 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 (Ribosomal Database Project) (Maidak *et al*., 1997), using the GDE multiple sequence editor program from RDP. Initial phylogenetic screening was constructed using the DNA distance program, Neighbour-Joining, in the PHYLIP package (Felsenstein, 1989) based on all SSU rDNA sequences of the bacterial groups, such as alpha, beta, gamma and delta *Proteobacteria*, Gram-positive bacteria and all of the other bacteria in RDP, plus the closely related SSU rDNA sequences which were absent from RDP. DNA distances were estimated by the method of Kimura (1980). Based on the initial phylogenetic results, appropriate subsets of SSU rDNA sequences were selected and subjected to final phylogenetic analysis by the maximum likelihood method with the program fastDNAml in RDP. Final phylogenetic trees were constructed with the transition/transversion ratio of 2·0 by the jumble option based on ten orderings of the data.

RESULTS

Specificity of the vector-targeted primers

Specific amplification of the SSU rDNA insert appeared to be obtained directly from transformant cells as well as from the purified plasmid DNA using the vector-specific primers. An approximately 1·6 kb fragment, the expected size of the cloned SSU rDNA insert plus the fragment from the vector, was amplified from a white colony and its plasmid DNA. An expected fragment of 80 bp was also amplified from a blue colony (data not shown). These results indicate that the designed primers are very specific to the pCR II vector under the amplified conditions used, and successful amplification directly from the transformant cells can be obtained.

To determine whether the amplified fragment originated from the SSU rRNA gene, DNA hybridization with the SSU rRNA gene probe was carried out. A Southern blot using a 1·1 kb internal SSU rDNA fragment from *Burkholderia cepacia* as a probe showed that the amplified fragment was from the SSU rRNA gene (data not shown). Since no fragment corresponding to a SSU rRNA gene was amplified from the blue colony which had no insert and no fragment was observed in the negative control, the possibility that the amplified SSU rRNA gene was from the *E. coli* genome or from contamination was ruled out.

SSU rDNA RFLP analysis

DNA was isolated from the surface tundra soil and SSU rRNA genes were amplified and cloned. A total of 123 colonies were obtained after they tested negative for α-complementation of β-galactosidase. While 69 (56·1 %) of these clones contained no detectable inserts, 54 clones (43·9 %) had an insertion detectable by PCR amplification with the vector-specific primers. A total of 43 of these clones contained the entire 1·5 kb SSU rDNA insert.

The clone library was screened for unique types by digestion with tetrameric restriction enzymes. A total of 27 patterns were obtained after digestion with restriction enzymes *Eco*RI and *Msp*I, and an additional 16 patterns were obtained from the undifferentiated clones with the restriction enzymes *Rsa*I and *Bst*VI. Thus, all of the 43 clones showed different RFLP patterns.

Phylogenetic analysis

To determine their phylogenetic diversity, all 43 clones were partially sequenced. While good sequences were obtained for most of the clones with the primer 790F, no sequence information was obtained for five of these clones (Table 1) These were then sequenced using the vector-specific primer TA106.

Phylogenetic analysis by the maximum likelihood method revealed the presence of three major and several minor groups that fell into several of the established lines of bacteria (Table 1). Most of the clones belong to the alpha (20·9 %) and delta (25·6 %) subdivisions of the *Proteobacteria* with lesser proportions in the beta (9·3 %) and gamma (4·7 %) subdivisions. About 10 % of the clones belong to Gram-positive bacteria, and 28 % of the clones were related to the deeply rooted phyla such as *Fibrobacter*, *Planctomyces*, spirochaetes, *Bacteroides* and green sulfur bacteria (Table 1). One clone could not be assigned to any described taxon. The results suggest that these clones are very diverse in phylogeny.

The majority of the clones (70 %) had sequences that were 5–15 % different from those in the current databases (Table 1). About 12 % of the clones differed by more than 15 % in sequence from the database, and 13 % of the clones differed by only 1–5 %. The results suggest that the sequences of these clones are also highly diverse.

One frequently encountered group was affiliated with the *Desulfuromonas* group of the delta subdivision of *Proteobacteria*, and consisted of clones S-3, S-12, S-13, S-21 and S-27 (Fig. 1). These clones were related to *Geobacter metallireducens* and *Pelobacter propionicus* with similarities of 87–91 % (Table 1). Clone S-46 also fell into the *Desulfuromonas* group based on the 5′ terminal sequence (Table 1). Another group within the delta subdivision consisted of clones S-34, S-40 and S-44. These clones branched deeply within the delta lineage (Fig. 1). Although clones S-40 and S-44 had higher similarities to *Pelobacter* and *Geobacter* species (Table 1), phylogenetic analysis by both distance matrix and maximum likelihood methods showed that these two clones were closely related to *Desulfosarcina* (Fig. 1).

The second abundantly represented group was in the alpha *Proteobacteria* and consisted of clones S-5, S-18,

Table 1. Diversity of the Siberian tundra soil SSU rDNA clones and sequences

Clone no.	Length of sequence (bp)	Corresponding to *E. coli* SSU rRNA gene	Group	Micro-organism with most similar sequences in the databases	Similarity (%)
S-1	229	868–1093	Alpha	*Rhodopseudomonas palustris*	93·9
S-2	257	861–1031	Delta	*Bdellovibrio bacteriovorus*	78·7
S-3	268	860–1041	Delta	*Geobacter sulfurreducens*	91·4
S-4	214	856–1068	Fibrobacter	*Acidobacterium capsulatum*	88·8
S-5	258	857–1110	Alpha	*Bradyrhizobium japonicum*	89·6
S-6	475		Gamma	*Azospirillum* sp. strain DSM 1712	93·8
S-7	266	856–1120	Fibrobacter	*Acidobacterium capsulatum*	86·4
S-8	253	857–1107	Beta	*Azoarcus* sp. strain S5b2	85·1
S-10	319	856–1176	Gram-positive	*Clavibacter michiganense*	95·9
S-11	253	878–1128	Beta	*Variovorax paradoxus*	90·3
S-12	249	873–1033	Delta	*Pelobacter propionicus*	87·4
S-13	256	860–1027	Delta	*Pelobacter propionicus*	90·2
S-14	260	28–318	Alpha	*Bradyrhizobium japonicum*	86·9
S-15	330	31–368	Planctomyces	Soil DNA clone MC100	90·3
S-16	322	888–1207	Spirochaetes	*Spirochaeta stenostrepta*	89·8
S-17	429		Gram-positive	*Heliobacterium chlorum*	78·6
S-18	352	857–1204	Alpha	*Bradyrhizobium* sp. strain LMG 9980	99·4
S-19	308	855–1162	Fibrobacter	*Holophaga foetida*	90·3
S-20	272	855–1127	Fibrobacter	*Holophaga foetida*	87·1
S-21	372	860–1147	Delta	*Pelobacter acetylenicus*	90·5
S-24	390	856–1244	Gram-positive	*Bacillus licheniformis*	85·2
S-25	249	857–1111	Alpha	*Bradyrhizobium* sp. strain LMG 9980	89·2
S-26	331	857–1188	Alpha	*Agrobacterium vitis*	88·7
S-27	307	861–1081	Delta	*Pelobacter propionicus*	87·8
S-28	308	857–1161	Alpha	*Rhizobium loti*	92·9
S-29	386		Gram-positive	Soil DNA clone MC 87	80·6
S-31	262	863–1036	Delta	*Polyangium cellulosum*	95·0
S-33	334	856–1188	Fibrobacter	Soil DNA clone MC27	93·6
S-34	311	860–1084	Delta	*Desulfosarcina variabilis*	91·6
S-35	272	857–1127	Fibrobacter	*Acidobacterium capsulatum*	91·9
S-36	349	857–1202	Green sulfur bacteria	*Chlorobium vibrioforme*	77·3
S-37	256	856–1114	Gram-positive	*Rhodococcus equi*	89·9
S-38	328	857–1187	Beta	*Burkholderia caryophylli*	92·0
S-39	262	857–1119	Bacteroides	*Cytophaga fermentans*	87·7
S-40	353	860–1126	Delta	*Pelobacter propionicus*	89·5
S-41	354	857–1213	Beta	*Zoogloea ramigera*	96·1
S-42	360	858–1215	Alpha	*Rhodopseudomonas palustris*	98·6
S-43	325	856–1179	Gamma	*Legionella steigerwaltii*	97·2
S-44	388	860–1161	Delta	*Geobacter sulfurreducens*	92·5
S-46	337	860–1103	Delta	*Desulfuromusa kysingii*	81·7
S-47	336	28–393	Alpha	*Bradyrhizobium japonicum*	91·9
S-48	306	1174–1492	Novel phylum	Unidentified soil bacteria	92·3
S-49	283	28–328	Fibrobacter	Soil DNA clone MC 26	93·4

S-25, S-42 (Fig. 2), S-47 (Table 1) and the more distantly related clone S-1. This group was closely affiliated with the *Bradyrhizobium* sub-group (89–99 % sequence homology) within the *Rhizobium–Agrobacterium* group. Clone S-14 could also belong to this group since it had about 87 % sequence homology for the first 300 bp of

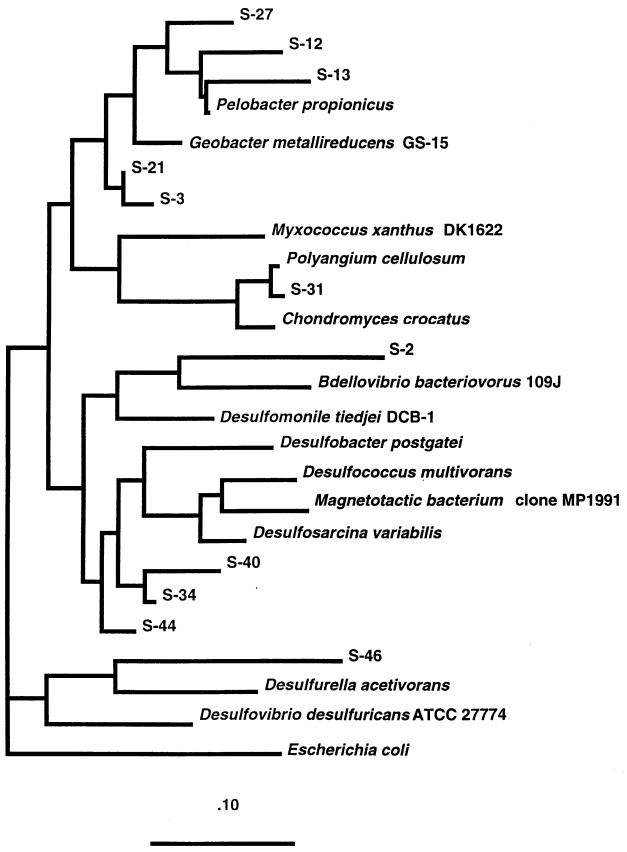

Fig. 1. Phylogenetic analysis of the rDNA clones affiliated with the delta subclass of the *Proteobacteria*. The tree was established by the maximum likelihood method based on the partial SSU rRNA gene sequences with *E. coli* as the outgroup.

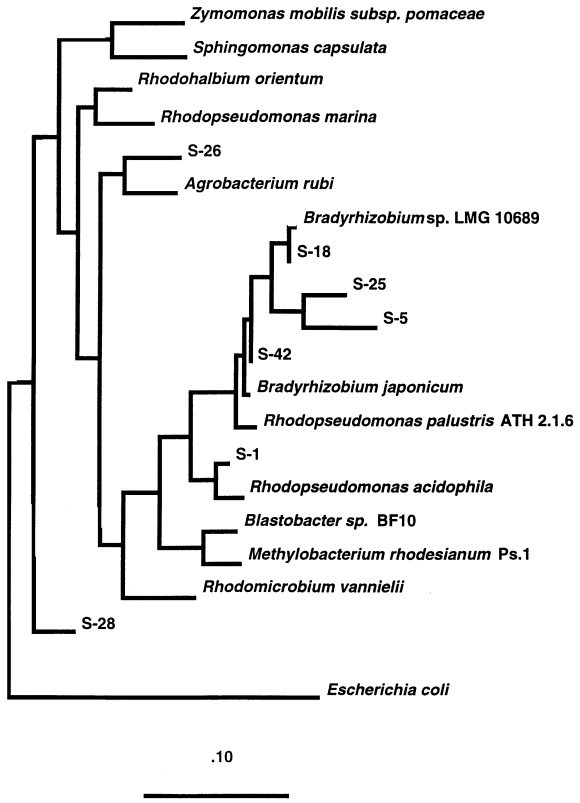

Fig. 2. Phylogenetic analysis of the rDNA clones affiliated with the alpha subclass of the *Proteobacteria*. The tree was established by the maximum likelihood method based on the partial SSU rRNA gene sequences with *E. coli* as the outgroup.

the SSU rDNA sequence to *Bradyrhizobium japonicum* (Table 1).

The third commonly encountered group comprised S-4, S-7, S-33 and S-35 and the more distantly related clones S-19 and S-20 (Fig. 3). This group was affiliated with the *Acidobacterium* subdivision of *Fibrobacter*. Clone S-49 also fell into this subdivision (Table 1). More interesting is that these clones showed distinct similarities (86–94%) to the sequences of the Australia soil rDNA clones MC 9, MC 13, MC 27 and MC 26, as well as *Acidobacterium capsulatum* and *Holophaga foetida* (Fig. 3, Table 1).

Another clone was affiliated with the *Planctomyces* group, and was closely related to the Australia soil rDNA clone MC 100 (Table 1). The affiliation with the Australian clones is interesting since that soil is dry and warm while the tundra is wet and cold.

Phylogenetic analysis showed that four clones fell into the beta subclass of the *Proteobacteria*, and are closely related to *Azoarcus* (S-8) and *Zoogloea* (S-41), both within the *Rhodocyclus* group, and *Variovorax paradoxus* (S-11) and *Burkholderia caryophylli* (S-38), both within the *Rubrivivax* group (Table 1, tree not shown). In addition, five clones were also affiliated with the Gram-positive bacteria, and showed a substantial degree of relatedness to *Clavibacter michiganensis* (S-10) within the *Arthrobacter* group, the Australian soil DNA clone MC 87 (S-29) of high-GC bacteria, *Bacillus licheniformis* (S-24) within the *Bacillus* group of the *Bacillus–Lactobacillus–Streptococcus* subdivision, S-17 within the *Desulfotomaculum* group, and *Rhodococcus equi* (S-37) within the *Mycobacterium* group of high-GC bacteria (Table 1, tree not shown). Only two clones were found within the gamma subdivision; they were closely related to *Azospirillum* (S-6) within the *Pseudomonas* group and *Legionella steigerwaltii* (S-43) within the *Legionella* group (Table 1).

DISCUSSION

No dominant RFLP patterns were found in the tundra soil because all 43 clones were different. Such a result is very similar to those observed in a Wisconsin soil (Borneman *et al*., 1996), but very different from those obtained at a hydrothermal vent and in an anaerobic hypolimnion of a lake (Moyer *et al*., 1994, 1995; J. Figueras and others, unpublished), habitats in which the diversity was much lower.

Phylogenetic analysis revealed the presence of 11 different groups of bacteria in the surface tundra soil,

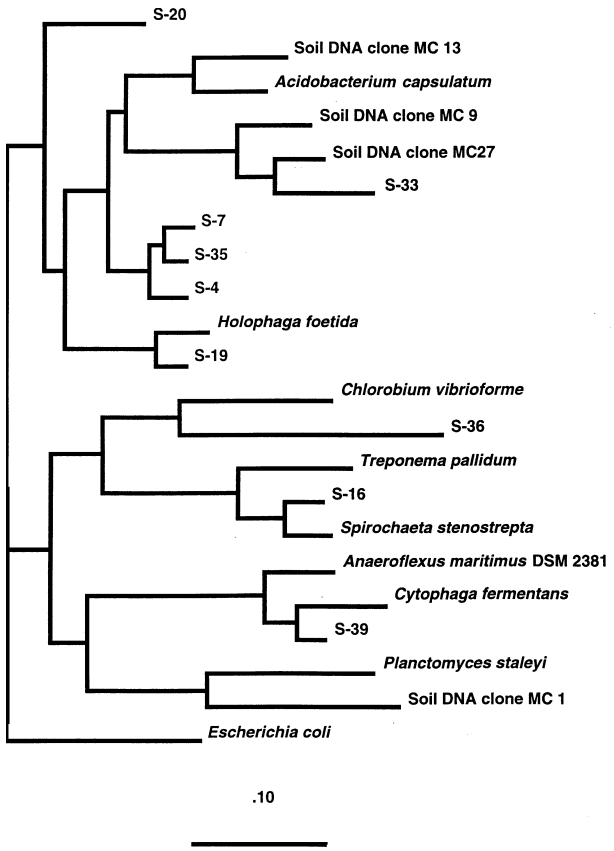

Fig. 3. Phylogenetic analysis of the rDNA clones affiliated with the deeply rooted phyla. The tree was established by the maximum likelihood method based on the partial SSU rRNA gene sequences with *E. coli* as the outgroup.

and the majority of the clones (87%) had sequences 5–25% different from the sequences in the current databases. These results suggest that these clones are very diverse in phylogeny and are suggestive of new genera or families. Most of the tundra soil bacterial community appears to never have been isolated and hence the physiology and function of these presumably dominant organisms are unknown.

Although the clones recovered were from a wide variety of phylogenetic groups, only a few clones (11·6%) fell into Gram-positive bacteria. This might have been due to ineffective lysis since we later found that the SDS-based high salt and temperature lysis method used here is not effective for some Gram-positive bacteria (Zhou *et al.*, 1996).

Microbial diversities have been analysed using the SSU rRNA gene sequence-based method with three soils and one sediment: Wisconsin agricultural soil (Borneman *et al.*, 1996), Australian subtropical soil (Liesack & Stackebrandt, 1992; Stackebrandt *et al.*, 1993), Japanese temperate soybean soil (Ueda *et al.*, 1995) and coastal marine sediments (Gray & Herwig, 1996). Our results are very different from the results from these soils and sediments. In the Wisconsin agricultural soil, the majority of the sequences obtained were from the *Cytophaga–Flexibacter–Bacteroides* group (21·8%), the low-GC Gram-positive bacteria (21·8%) and *Proteobacteria* (16·1%). Within the *Proteobacteria*, the authors found more clones in the beta subdivision (8·1%) than in the alpha (1·6%) and delta (3·2%) subdivisions. In contrast to the Wisconsin soil, most of the phylotypes in the tundra soil sample were members of the *Proteobacteria* (60·5%) and *Fibrobacter* (16%), and only one clone (2·3%) from the *Cytophaga–Flexibacter–Bacteroides* group. Within the *Proteobacteria*, the majority of the clones in the tundra soil was from the delta (25·6%) and alpha (20·9%) subdivisions, which are most related to sulfate-reducing or nitrogen-fixing bacteria, rather than from the beta subdivision. Such differences can most likely be explained by differences in temperature, moisture, pH and vegetation. The water-saturated condition of the tundra during the thawed period probably creates anaerobic conditions which may explain the high proportion of clones from the delta *Proteobacteria*, including many sulfur-reducers.

The results from our tundra soil and the Australian subtropical soil are not directly comparable since one of the PCR primers used in the Australian study was designed to amplify streptomycete rDNA (Stackebrandt *et al.*, 1993), rather than eubacteria, which we used. Nonetheless, the alpha *Proteobacteria* was a dominant group in both the Siberian and Australian soils. The major groups in the Japanese soybean soil were very different from the tundra soil. A deeply rooted group, perhaps related to the low-GC Gram-positives, was the most dominant in the Japanese soil and a high-GC Gram-positive group was the second most abundant. The major groups in the coastal marine sediments were also very different from the tundra soil. The Gram-positive bacteria were the most abundant in the coastal marine sediments and the gamma *Proteobacteria* were the second most abundant.

Because of the very high SSU rDNA diversity that seems to be prevalent in soils, comparisons among different microbial communities can only be preliminary; the sample size is probably still too small. Nonetheless, it does provide initial information which already suggests that the composition is much more novel than we expected.

ACKNOWLEDGEMENTS

This project was supported by the Center for Microbial Ecology and the National Science Foundation, grant no. DEB9120006.

REFERENCES

Borneman, J., Skroch, P. W., O'Sullivan, K. M., Palus, J. A., Rumjanek, N. G., Jansen, J. L., Nienhuis, J. & Triplett, E. W. (1996). Molecular microbial diversity of an agricultural soil in Wisconsin. *Appl Environ Microbiol* **62**, 1935–1943.

Devereux, J., Haeberli, P. & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12**, 387–395.

Dunican, L. K. & Rosswall, T. (1974). Taxonomy and physiology of tundra bacteria in a relation to site characteristics. In *Soil Organisms and Decomposition in Tundra*, pp. 79–92. Edited by A. J. Holding, O. W. Heal, S. F. Maclean, Jr & P. W. Flanagan. Stockholm: IBP Tundra Biome Steering Committee.

Felsenstein, J. (1989). PHYLIP – Phylogeny inference package (Version 3.2). *Cladistics* **5**, 164–166.

Gray, J. P. & Herwig, R. P. (1996). Phylogenetic analysis of the bacterial communities in marine sediments. *Appl Environ Microbiol* **62**, 4049–4059.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kwok, S. & Higuchi, R. (1989). Avoiding positives with PCR. *Nature* **339**, 237–238.

Liesack, W. & Stackebrandt, E. (1992). Occurrence of novel groups of the domain *Bacteria* as revealed by analysis of genetic material isolated from an Australian terrestrial environment. *J Bacteriol* **174**, 5072–5078.

Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1997). The RDP (Ribosomal Database Project). *Nucleic Acids Res* **25**, 109–111.

Moyer, C. L., Dobbs, F. C. & Karl, D. M. (1994). Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl Environ Microbiol* **60**, 871–879.

Moyer, C. L., Dobbs, F. C. & Karl, D. M. (1995). Phylogenetic diversity of the bacterial community from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl Environ Microbiol* **61**, 1551–1562.

Stackebrandt, E., Liesack, W. & Goebel, B. M. (1993). Bacterial diversity in a soil sample from a subtropical Australian environment as determined by 16S rDNA analysis. *FASEB J* **7**, 232–236.

Ueda, T., Suga, Y. & Matsuguchi, T. (1995). Molecular phylogenetic analysis of a soil microbial community in a soybean field. *Eur J Soil Sci* **46**, 415–421.

Weisburg, W. W., Barns, S. M., Pelletier, D. A. & Lane, D. J (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.

Zhou, J.-Z., Fries, M. R., Chee-Sanford, J. & Tiedje, J. M. (1995). Phylogenetic analysis of a new group of denitrifiers capable of anaerobic growth on toluene: description of *Azoarcus tolulyticus* sp. nov. *Int J Syst Bacteriol* **45**, 500–506.

Zhou, J.-Z., Bruns, M. A. & Tiedje, J. M. (1996). DNA recovery from soils of diverse composition. *Appl Environ Microbiol* **62**, 461–468.

Received 12 August 1997; accepted 26 August 1997.