Extremophiles (2001) 5:23–33
Digital Object Identifier (DOI) 10.1007/s007920000169

ORIGINAL PAPER

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16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica

Received: July 31, 2000 / Accepted: October 20, 2000 / Published online: February 1, 2001

Abstract The prokaryotic diversity of aerobic and anaerobic bacterial isolates and of bacterial and archaeal 16S rDNA clones was determined for a microbial mat sample from the moated region of Lake Fryxell, McMurdo Dry Valleys, Antarctica. Among the anaerobic bacteria, members of *Clostridium estertheticum* and some other psychrotolerant strains dominated whereas methanogens and other Archaea were lacking. Isolates highly related to *Flavobacterium hibernum*, *Janthinobacterium lividum*, and *Arthrobacter flavus* were among the aerobic bacteria most frequently isolated. Assessment of more than 350 partial 16S rDNA clone sequences of libraries generated by Bacteria- and Archaea-specific PCR primers revealed a rich spectrum of bacterial diversity but only two different archaeal clone sequences. Among the Bacteria, representative sequences belonged to the class *Proteobacteria*, order *Verrucomicrobiales*, class *Actinobacteria*, *Clostridium/Bacillus* subphylum of Gram-positives, and the *Cytophaga-Flavobacterium-Bacteroides* phylum. The clones formed about 70 higher taxonomy groups (<98% sequence similarity) and 133 potential species, i.e., groups of clones sharing greater than 98% similarity. Only rarely were clone sequences found to be highly related to Lake Fryxell isolates and to strains of described species. Subsequent analysis of ten sequencing batches of 36 individual clones indicated that the diversity might be still higher than had been assessed.

Key words Biodiversity · Lake Fryxell mat · Antarctica · Psychrophiles

Communicated by G. Antranikian

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Introduction

The search for novel and biotechnologically exploitable organisms (Ashbolt 1990) has motivated microbiologists to screen the largely unexplored Antarctic continent and surrounding marine sites. Although studies started some decades ago (Pfister 1965; Marshall 1966; Tsyganov 1970; Friedmann 1980), it was not until the early 1990s that more emphasis was placed on the description of new genera and species. The sites of these new taxa ranged from freshwater lakes (Franzmann et al. 1991, 1997; McCammon et al. 1998), saline and hypersaline lakes and ponds (Dobson et al. 1993; Bowman et al. 1997d; Mountfort et al. 1988; Labrenz et al. 1998), soil and sandstone (Bowman et al. 1996; Schumann et al. 1997; Suzuki et al. 1997; Hirsch et al. 1998), and sea ice (Bowman 1998; Bowman et al. 1997a, e; Junge et al. 1998) to the coastal region (Bozal et al. 1997) and the marine environment (Irgens et al. 1996; Gosink et al. 1998; Bowman et al. 1997c). In addition, selected Antarctic environmental sites were subjected to more general microbial ecological studies, focusing on the seasonal and spatial variability and distribution of prokaryotes in coastal waters and sea ice (Gosink and Staley 1995; Maugeri et al. 1996; Murray 1998; Bowman et al. 1997a, 1997b, 1997c), soil (Friedmann 1980), glaciers (Abyzov et al. 1998), and lakes (Kriss 1976; Osnitskaia 1978; Priscu 1998).

The introduction of culture-independent molecular screening techniques has allowed microbiologists to examine a facet of microbial diversity not necessarily reflected by the results of culturing studies. None of the approaches currently used to assess diversity can claim to be more efficient than another. Within a given time period and despite the known methodological bias involved in the generation of 16S rDNA clone libraries (von Wintzingerode et al. 1997), 16S rDNA sequences allow the assessment of a broader range of diversity than that obtained by cultivation studies. These sequences, on the other hand, despite the inability of ex situ cultivation to mirror in situ growth conditions, permit us at least to reflect somewhat on the physiological role of isolates in their environmental niche.

Certainly, the synergistic effect of both approaches applied in parallel to a natural sample cannot be overestimated in understanding the traits that have made clones/isolates successful.

In this article, we report on the molecular analysis of a sample from a microbial mat from Lake Fryxell, Antarctica, thus extending studies on lakes from other regions of the continent. Results of sequence analysis of 16S rDNA clone libraries for Archaea and Bacteria, which are compared to results of culture independent studies from other Antarctic lakes and to the diversity of strains obtained from cultivation studies of the same mat sample, point toward a rich diversity of prokaryotic taxa.

Materials and methods

Source of sample

A mat sample was retrieved from the shallow, moated area of Lake Fryxell, McMurdo Dry Valleys region, Antarctica, 77°36′ S, 162°6′ E, in February 1999. The sample was shipped at 5°C to the University of Nottingham; from there it was distributed frozen to the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ).

Media and cultivation

The cultivation of aerobes and anaerobes centered on the use of a range of different media and growth temperatures. Aerobic cultures were directly plated after homogenizing the mat sample and plating from a tenfold dilution series. Media were selected that were biased toward oligotrophs, actinobacteria, and planctomycetes. Incubation temperatures were 5°, 15°, and 25°C. In addition, enrichment methods were used as described for the selection of budding and appendaged bacteria. More than 900 isolates were obtained, of which a subset of 100 strains were the subject of a previous publication (Tindall et al. 2000). For the isolation of anaerobic strains, about 1 cm^3 of the sample was suspended in anaerobic DSMZ medium 119 (DSMZ 1998) (H_2+CO_2 atmosphere) and distributed to the following growth media:

A: DSMZ medium 119 (H_2+CO_2 atmosphere) plus yeast extract (1 g l^{-1}) without (A-1) and with antibiotics (300 mg ml^{-1} penicillium G and 100 mg ml^{-1} cycloserine) (A-2), at 4°C and 20°C

B: DSMZ medium 119 (N_2+CO_2 atmosphere) plus methanol (0.5% v/v) without and with antibiotics (300 mg ml^{-1} penicillium G and 100 mg ml^{-1} cycloserine), at 20°C

C: DSMZ medium 63 (N_2 atmosphere), at 10°C

D: Half-concentrated Wilkins–Chalgren broth (WIC) (1805-17; Difco, Detroit, MI, USA)

Following inoculation, half the tubes (D-1) were pasteurized at 10°C: the other half (D-2) were not

Growth was observed after 2 to 4 weeks in media A-1, C, and D, and the following media were inoculated:

From DSMZ medium 119, prepared without antibiotics (A-1)
- DSMZ medium 63, incubated anaerobically (A-1/C-an)
- DSMZ medium 63 modified, omitting $FeSO_4$, sodium thioglycolate, and ascorbic acid, but supplemented separately with 1 g l^{-1} trypticase and 1 g l^{-1} glucose after sterilization, incubated aerobically (A-1/C-aer)

From DSMZ medium 63 (C)
- DSMZ medium 63, cultivated anaerobically (C/C-an)
- DSMZ medium 63 modified (see above), cultivated aerobically (C/C-aer)

From WIC, pasteurized (D-1)
- WIC, cultivated anaerobically (D-1/D-an)

From WIC, nonpasteurized (D-2)
- WIC, cultivated anaerobically (D-2/D-an)

Pure cultures were obtained after growth on respective agar media by picking colonies and restreaking.

Isolation of DNA

Four times, 1 g frozen mat material from adjacent locations was blended separately with each 9 ml phosphate buffer (0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, pH 7.4) in a Stomacher Lab Blender (Seward Medical, London, UK) (Jay 1979; Cambell and Greaves 1990) for 60 s at 4°C. Released organisms were combined, the supernatant was prefiltered through a 5-µm nitrocellulose filter (Sartorius, Göttingen, Germany), and microorganisms were collected by centrifugation in 1.5-ml portions at 16,000 *g* for 5 min. These pellets were individually suspended in 400 µl saline EDTA (0.15 M NaCl, 10 mM EDTA, pH 8.0) and used immediately for the extraction of DNA.

Isolation of DNA from pure cultures and organisms recovered from mat samples followed the method described by Rainey et al. (1996). Some minor modifications were introduced during DNA isolation from mat samples. In procedure 1, one-half of the cells were lysed with 20 µl lysozyme (5 mg lysozyme ml^{-1}) for 45 min at 37°C and incubated with proteinase K and sodium dodecylsulfate (SDS) for 60 min at 55°C. In procedure 2, the other half of the cells were lysed with 30 µl lysozyme and 10 µl proteinase K for 45 min at 37°C, followed by repeated freeze-and-thaw cycles (three times for 10 min at –70°C and three times at 55°C). The DNA was then purified by Prep-A-Gene treatment (BioRad, Munich, Germany). Samples of procedures 1 and 2 were combined for subsequent PCR amplification.

Amplification of 16S rRNA genes and cloning of PCR products

Amplification of the almost complete 16S rRNA genes was carried out from genomic DNA by PCR as described by Rainey et al. (1996). For the generation of a clone library of

members of the domain Bacteria, the forward primer GAGTTTGATCCTGGCTCAG corresponded to positions 10–30 of *Escherichia coli* 16S rDNA (Brosius et al. 1978), whereas the reverse primer AGAAAGGAGGTGATCCAGCC corresponded to the complement of positions 1523–1542. For the generation of a clone library of members of the domain Archaea, the forward primer ArcF TCCGGTTGATCCTGCC corresponded to positions 10–30 of *Escherichia coli* 16S rDNA, whereas the reverse primer GGGTCTCGCTCGTTACC corresponded to the complement of positions 1,084–1,100. Cloning of PCR products was done with the pGM-T Vector system II (Promega, Heidelberg, Germany) following the manufacturer's instructions.

Reamplification of the cloned 16S rDNA PCR products

Crude lysates of clones were generated by suspending individual colonies in 100 µl water followed by extraction of DNA as described by Sambrook et al. (1989). PCR fragments of clones were reamplified by PCR with M13 PCR primers (M13–20 forward GTAAAACGACGGCCAGT and M13 reverse GGAAACAGCTATGACCATG). PCR conditions followed described procedures. The length of amplified products was determined against marker Boehringer III (Boehringer, Mannheim, Germany). PCR products were purified by Quiagen DNA purification kit (Quiagen, Hilden, Germany).

Sequence analysis

Partial sequence analysis of 16S rDNA, using the reverse primer CTGCTGCCTCCCGTA and CCCCGTAGGGCCT/CGG (positions 343–357) for members of the domains Bacteria and Archaea, respectively, followed the procedure described by Rainey et al. (1996).

Hybridization of cloned 16S rDNA fragments

The reamplified 16S rDNA clone fragments of the Archaea-specific clone library were hybridized with probes. Hybridization and detection followed published methods (Pukall et al. 1998).

Analysis of sequence data

The sequences were initially analyzed using the BLAST search facility (www.ncb.nlm.nih.gov/blast/blast.cgi) and the RDPII analysis software (www.cme.msu.edu/RDP/html/analyses.html). Some sequences were included in the ARB software (www.mikro.biologie.tu-muenchen.de) to search for the closest evolutionary relative. All sequences were then aligned manually against the DSMZ database of 16S rDNA sequences. Detailed analysis was performed with sequences related to *Clostridium estertheticum* applying the least squares distance method algorithm of DeSoete (1983), in which similarity values were transformed into phylogenetic distances values that compensate for multiple substitutions at any given site (Jukes and Cantor 1969). All analyses were done on a SUN SparcII workstation.

Results

Isolation and 16S rDNA sequence analysis of strains

Direct plating or enrichment methods were used in the isolation of anaerobes and aerobes. Slowly growing colonies, which emerged after enrichment under anaerobic conditions, were subcultivated under anaerobic and aerobic conditions. Medium 119, supplemented with antibiotics and methanol, did not support growth of any organisms, indicating the absence of methanol-utilizing cells. Microscopic examination of mat samples for the presence of cells showing the typical green fluorescence of methanogens indicated that these organisms were not present in large numbers. However, analysis of the archaeal 16S rDNA clone library (see following) showed that two different types of methanogens were actually found to be part of the community of prokaryotes. Because these organisms are extremely sensitive to oxygen, one must assume that a low redox potential was not maintained during transport of the mat sample from Lake Fryxell to the DSMZ via Nottingham, UK.

Anaerobic enrichment cultures were subcultured under anaerobic and aerobic conditions, resulting in the isolation of 17 strains from the domain Bacteria. These cultures were affiliated to described taxa by comparative 16S rDNA analyses (Table 1). For rapid taxonomic assessment, partial 16S rDNA sequences (350 nucleotides from the 5′-terminus) were determined and the almost complete sequence analyzed for those sequences that showed less than 99% similarity to the sequence of an organism deposited in public databases. The majority of strains belong to the *Clostridium/Bacillus* subphylum of gram-positive organisms (9 strains) whereas the others cluster with the *Actinobacteria* (4 strains), β- and γ-*Proteobacteria* (1 and 3 strains, respectively), and *Flavobacterium* (1 strain).

The more than 800 strains cultivated under the aerobic enrichment/isolation strategy were subjected to a sorting strategy by Fourier-transformed-infrared (FTIR) spectroscopy. Some results, including about 100 strains, have been published (Tindall et al. 2000). Based upon FTIR heterogeneity of 20, these strains formed about 21 single-strain lineages and 9 multistrain clusters (Table 2). To determine the phylogenetic relatedness of strains forming either clusters or single-strain lineages, the isolates were subjected to partial 16S rDNA sequence analysis. The majority of strains belonged to *Janthinobacterium lividum* (8 strains), *Flavobacterium hibernum* (7 strains), and *Arthrobacter flavus* (6 strains), each of which shared intracluster similarities of 100%. The degree of relatedness between the isolates sequences to date and type strains of described species was

Table 1. Strains from a mat sample of Lake Fryxell, Antarctica, originating from anaerobic enrichment

Culture conditions/strain number	Isolation temperature (°C)	Nearest phylogenetic neighbor	16S rDNA similarity (%)	Phylum or subphylum
C/C-aer/c	8	*Janthinobacterium lividum*[a]	100	*β-Proteobacteria*
C/C-aer/a,	8	*Pseudomonas veronii*[a]	99.9	*γ-Proteobacteria*
D-2/D-an/IV	8			
A1/C-aer/OII	8	*Psychrobacter glacincola*	98.2	*γ-Proteobacteria*
A-1/C-an/E	8	*Frigoribacterium faeni*	95.5	*Actinobacteria*
D-2/D-an/III	8			
A-1/C-an/z-K1	8[b]			
C/C-aer/b	8	*Exiguobacterium acetylicum*	97.9	*Clostridium-Bacillus*
A1/C-aer/OI	8	*Cryobacterium psychrophilum*	96.5	*Clostridium-Bacillus*
D-1/D-an/II	8	*Clostridium estertheticum*	99.5	*Clostridium-Bacillus*
C/C-an/B1	8	*Clostridium estertheticum*	99.4	*Clostridium-Bacillus*
A-1/C-an/C1	8	*Clostridium estertheticum*	98.6	*Clostridium-Bacillus*
A-1/C-an/I	8	*Clostridium estertheticum*	97.5	*Clostridium-Bacillus*
A-1/C-an/z-K2	8[b]	*Carnobacterium alterfunditum*	99.9	*Clostridium-Bacillus*
A1/C-aer/OIII	8[b]	*Carnobacterium alterfunditum*	99.9	*Clostridium-Bacillus*
A-1/C-an/z-K3	8	*Clostridium vincentii*	99.4	*Clostridium-Bacillus*
AM119an/H_2+CO_2	20	*Acetobacterium bakii*	98.5	*Clostridium-Bacillus*
A1/C-aer/OIV	8	*"Flavobacterium xylanivorum"*	98.0	Flavobacterium

[a]Identical sequence to those isolated under anaerobic conditions
[b]Strains were first enriched at 8°C but subsequently isolated at 15°C

Table 2. Strains from a mat sample of Lake Fryxell, Antarctica, originating from aerobic enrichment

Strains	Nearest phylogenetic neighbor	Similarity (%)	Phylum or subphylum
1.10	*Bosea thiooxydans* ("Afipia" group 9)	97.6	*α-Proteobacteria*
4.10	*Pseudomonas* sp. 51, unclassified bacterium T22	100	*β-Proteobacteria*
4.11, seven others	*Janthinobacterium lividum*[a]	100	*β-Proteobacteria*
14.15	*Duganella zoogloeoides*	93.9	*β-Proteobacteria*
7.31, 11.19	*Pseudomonas veronii*[a]	99.9	*γ-Proteobacteria*
4.21	*Psychrobacter glacincola*	97.4	*γ-Proteobacteria*
4.5, six others	*Flavobacterium hibernum*	97.3	*Flavobacterium*
4.31a	*Micrococcus luteus* HN2—11	100	*Actinobacteria*
5.39	*Micrococcus luteus* HN2—11	99.7	*Actinobacteria*
6.20, five others	*Arthrobacter flavus*	100	*Actinobacteria*
10.9	*Isosphaera pallida* Schlesner strain 657	82.7, 88.6	*Planctomycetes*

[a]Identical sequence to those isolated under aerobic conditions

mostly greater than 96%, except for a strain related to *Duganella zoogloeoides* (93.9%), a novel representative of the *Planctomycetales* sharing less than 90% similarity with a German planctomycete isolate (Griepenburg et al. 1996), and *Isosphera pallida,* a thermophilic planctomycete (82.7% similarity). The taxonomic evaluation of other aerobic isolates is presently under investigation (Tindall et al., unpublished).

Analysis of 16S rDNA clone libraries

Bacteria

About 378 clone inserts were partially sequenced, 325 of which were found to contain the amplified 16S rDNA fragment (320 nucleotides from the 5′-terminus). This stretch contains variable regions (positions around 100 and 200), which not only discriminate well between closely related species but in most cases will also underestimate the degree of sequence similarity of almost complete sequences by 1%–12% for strains belonging to different phyla. At the intraphylum level, individual species show as much as 8% differences because in different taxa the variable regions are located at different regions of the 16S rDNA molecule (Stackebrandt and Rainey 1995). For some pairs of organisms belonging to different phyla, similarities based upon partial sequences are slightly higher than those based upon almost complete sequences (e.g., *E. coli* and *Cytophaga hutchensonii*). With a single exception, the similarity values determined for a small portion only of the molecule may

differ significantly among taxa, so we have refrained from showing a phylogenetic tree of the clone sequences and the cultured organisms for which the highest matching similarity has been scored.

Table 3 (column 4) lists the percentage similarities between clones or representatives of clone groups and the sequence of a described species or, in some cases, deposited clone sequences generated in another molecular environmental study. Analysis using BLAST, RDP, ARB, and DSMZ databases of 16S rDNA sequences agree in those cases in which the different databases contain the same selection of reference sequences. Comparing clone sequences and the 16S rDNA of its closest currently cultured relative, the range is between 77.9% and 100%. A group of clone sequences that form an exclusive 16S rDNA sequence cluster with its nearest neighbor is termed a clone cluster (Table 3 , column 3). The number of clones is shown in column 4, which also indicates, in parentheses, the number of individual potential species (see following), defined as clone sequences that share greater than 98% similarity (column 5). This method may well underestimate the actual number of species (Stackebrandt and Goebel 1994) but serves as a working hypothesis.

The range of similarity values of partially sequenced 16S rDNA of type strains belonging to different subclasses of *Proteobacteria*, Gram-positive bacteria, and the *Cytophaga-Flavobacteria-Bacteroides* (CBF) complex is generally less than 80%, whereas within these higher taxonomic clusters the values are generally greater than 80%. It is obvious from the data shown in Table 3 that most sequences fall into the radiation of the higher taxa of *Proteobacteria*, Gram-positive bacteria, CBF, and *Verrucomicrobiales*. Within each higher taxon, different sublines are represented, such as four of the five subclasses of the class *Proteobacteria*, low- and high G+C mol% Gram-positive bacteria (*Clostridium/Bacillus* and *Actinobacteria*, respectively), and each of the three main genera of the CBF complex. These clone clusters may contain subgroups of clones sharing greater than 98% similarity. The compilation in Table 3 indicates that many clone sequences are very similar to the sequence of species of described genera, although only the highest score is presented. Affiliations below 80% (*Acidomicrobium ferrooxidans, Clavibacter michiganense, Stigmatella aurantiaca*) are considered highly tentative and only given because different search methods agree, by and large, in their analysis.

Although not always deducible with a high degree of confidence, the affiliation of clone sequences to those of described taxa indicates the presence of anaerobic and aerobic species. The anaerobes are mainly affiliated to genera of the *Clostridium/Bacillus* subphylum (136 clones), e.g., *Clostridium*, *Eubacterium, Sporomusa, Ruminococcus, Desulfosporosinus, Acidaminobacter,* and *Acetobacterium,* as well as to the *Bacteroides* subphylum (59 clones). Others originate from the facultative anaerobes *Rhodopseudomonas* (α-*Proteobacteria*) as well from the obligate anaerobes *Desulfomonile* and *Pelobacter* (σ-*Proteobacteria*). Aerobic clones originate mainly from members of the taxa *Proteobacteria*, *Cytophaga*, *Flavobacteria*, and *Verrucomicrobiales*. In some cases the closest relatives of the Antarctic clone sequences are other clone sequences and uncultured bacteria, e.g., of the order *Verrucomicrobiales*. As observed among the cellular organisms, some of these relatives have been reported to originate from the Antarctic continent.

On the basis of complete 16S rDNA sequences, a binary similarity value of 97.5% indicates the presence of two distinct species (Stackebrandt and Goebel 1994). As differences in similarity values between partial and almost complete sequences are less significant for highly related sequences than for unrelated organisms, it is assumed here that the level of about 98% similarity to its nearest neighbor signals the presence of at least one potential new species thriving in the mat sample. In contrast to the situation found among cultured strains, in which about half the strains represent potentially new species, the vast majority of clone sequences appear to represent novel species. Exceptions are clones related to *Chelatobacter heintzii*, *Rhodoferax antarcticus*, *Aquaspirillum delicatum*, and *Clostridium estertheticum*.

The 325 sequences obtained belong to 133 potential species, the majority of which are represented only once (76 clones) or twice (32 clone pairs). Potential species containing a high number of clone sequences are much less frequent, such as those related to *Bacteroides forsythus* (48 sequences), *Acetonema longum* (25), *Rhodoferax fermentans* (22), and the uncultured bacterium CO19 (16). Figure 1 displays the diversity of 8 phylospecies and 4 isolates that are most closely related to *C. estertheticum* and other members of *Clostridium* group If (Stackebrandt and Hippe 2000). Analysis of ten sequencing gels, containing 36 clones each, allows an estimation of whether the sequences of 325 clones examined give an approximation of the phylogenetic diversity of the clone library. Figure 2 displays the increase in the diversity of clone clusters revealed by the analysis of each individual batch of 36 sequences as compared to the number of clone clusters of the previous batch. Although the first gel indicated the presence of 20 individual clone clusters, between 1 and 10 novel clone clusters were detected subsequently with each new batch. Thus, it can be assumed that the diversity is even higher than that determined by the exploration of 70 clone clusters (Table 3), and it is significantly higher when potential species are used as a measure of diversity.

Archaea

Analysis of 72 clones of a clone library generated with Archaea-specific primers revealed the presence of two different phylotaxa, the majority of clones being distantly related to *Methanoculleus palmolei* (89%–92%), whereas the others were related to the Antarctic clone sequence Ant 12 antarctic clone 371 (Table 3). A probe specific for *M. palmolei/M. olentangyi* was synthesized and hybridized against 319 clones of the library. More than 90% of the clones could be affiliated to this and possibly closely related taxa, indicating the restricted diversity of amplified archaeal rDNA sequences. The fact that the mat sample was filtered through a 5-μm filter suggests that methano-

Table 3. Phylogenetic affiliation of partial 16S rDNA clone sequences, the frequency of occurrence in the clone library, and range of intraclone cluster similarity

Representative clone of clone cluster and 16S rDNA accession numbers	Nearest phylogenetic neighbor	Range of similarity to nearest neighbor (%)	Number of clones per cluster (number of potential species)	Range of intraclone cluster similarity (%)
BACTERIA				
α-*Proteobacteria*				
161ev, AJ287618	*Chelatobacter heintzii*	99.3	1	
30ev, AJ287619	*Paracoccus versutus*	94.6	5 (1)	100
250ev, AJ287620	*Rhodobacter capsulatus*	96.6	1	
384ev, AJ287621, AJ287622	*Holospora obtusa*	83.3–86.4	2 (2)	81.9
144ev, AJ287623	*Sphingomonas natatoria*	90.7–96.4	5 (3)	94.5–100
58ev, AJ287624	*Sphingomonas suberifaciens*	96.9	1	
417ev, AJ287625	*Sphingomonas asaccharolytica*	95.0	1	
407ev, AJ287626	*Caulobacter vibrioides*	85.7	1	
423ev, AJ287627	*Rhodopseudomonas acidophila*	86.9	1	
251ev, AJ287628	*Hyphomonas oceanitis*	88.7	1	
322ev, AJ287629	*Pedomicrobium australicum*	87.5	1	
β-*Proteobacteria*				
222ev, AJ287635	*Rhodoferax fermentans*	95.7–97.1	22 (1)	98.0–100
184ev, AJ287646	*Rhodoferax antarcticus*	98.7–100	6(1)	98.7–99.4
101ev, AJ287636	*Propionvibrio dicarboxylicus*	96.4	3 (1)	100
183ev, AJ287637, AJ287638	*Nitrosospira multiformis*	86.5–88.4	2 (2)	91.2
255ev, AJ287639	*Ideonella dechloratans*	95.6	1	
350ev, AJ287640	*Polaromonas vacuolata*	92.5	2 (1)	100
394ev, AJ287641	*Aquaspirillum delicatum*	97.8	1	
γ-*Proteobacteria*				
187ev, AJ287655	*Xanthomonas albilineans*	80.5	1	
245ev, AJ287656	*Legionella rubrilucens*	92.7–95.8	2 (1)	92.4
328ev, AJ287658	*Methylocaldum szegediense*	86.5	1	
σ-*Proteobacteria*				
305ev, AJ287630	*Bdellovibrio bacteriovorus*	86.8	1	
244ev, AJ287631	*Pelobacter propionicus*	95.8	2 (1)	100
123ev, AJ287632	*Stigmatella aurantiaca*	79.9	2 (1)	100
265ev, AJ287633	*Myxococcus xanthus*	86.8	1	
86ev, AJ287634	*Desulfomonile tiedjei*	80.6	1	
Verrucomicrobiales				
47ev, AJ287649	Mc17	82.7–83.1	2 (1)	99.0
90ev, AJ287647, AJ287648, AJ287650	*Verrucomicrobium spinosum*	80.9–82.7	3 (2)	83.9–98.4
15ev, AJ287651, AJ287652	CO19, uncultured bacterium	85.0–91.0	17 (2)	88.4–100
200ev, AJ287653	LD19, unidentified bacterium	85.3	1	
49ev, AJ287654, AJ287659	LCK 04, uncultured freshwater bacterium	80.9–85.9	14 (2)	94.8–100
Cytophaga-Flavobacteria-Bacteroides				
122ev, AJ287664, AJ287665, AJ287676-AJ2878	*Bacteroides forsythus*	83.9–86.6	58 (4)	92. 6–100
65ev, AJ287667	*Bacteroides splanchnicus*	81.6	1	
22ev, AJ287675	*Cytophaga hutchinsonii*	84.4–84.8	2 (1)	98.5
429ev, AJ287662, AJ287663	*Flavobacterium ferrugineum*	87.5–87.8	2 (2)	93.9
421ev, AJ287674	*Flavobacterium hibernum*	92.8	1	
240ev, AJ287673	*Flavobacterium johnsoniae*	93.8	1	
168ev, AJ287661	*Flectobacillus major*	87.5	1	
204ev, AJ287671, AJ287672	*Flexibacter aurantiacus*	91.5–94.2	2 (2)	90.1
61ev, AJ287668, AJ287669	LCK 73, uncultured marine bacterium	88.2–96.0	10 (2)	89.6–99.7
87ev, AJ287670	*Marinilabilia agarovorans*	81.3	1	
Clostridium-Bacillus subline				
306ev, AJ287704, AJ287746, AJ287747	*"Acetivibrio agarovorans"*	84.5–88.6	3 (2)	81.6–82.6
303ev, AJ287708	*Acetobacterium bakii*	97.0–97.3	2 (1)	99.7
414ev, AJ287709	*Acetobacterium woodii*	96.5	1	
108ev, AJ287698-AJ287700	*Acetonema longum*	85.7–89.2	29 (4)	93.5–100
355ev, AJ287712-AJ287715	*Acidaminobacter hydrogenoformans*	82.4–88.0	7 (4)	86.3–98.8

Table 3. *(Continued)*

Representative clone of clone cluster and 16S rDNA accession numbers	Nearest phylogenetic neighbor	Range of similarity to nearest neighbor (%)	Number of clones per cluster (number of potential species)	Range of intraclone cluster similarity (%)
248ev, AJ287703	*Caloramator indicus*	85.3	1	
146ev, AJ287687		89.7	1	
400ev, AJ287732-AJ287739	*Clostridium estertheticum*	93.8–97.5	14 (8)	93.2–100
105ev, AJ287723-AJ287729	*Clostridium fallax*	91.0–93.2	12 (5)	84.9–100
29ev, AJ287681	*Clostridium lentocellum*	86.9	2 (1)	98.5
274ev, AJ287721	*Clostridium limosum*	92.0	1	
92ev, AJ287730, AJ287731	*Clostridium subterminale*	90.0–91.0	2 (2)	95.0
98ev, AJ287707	*Clostridium termitides*	94.2	1	
10ev, AJ287706, AJ287748, AJ287749	*Clostridium viride*	83.1–85.5	5 (4)	82.5–99.1
82ev, AJ287692 AJ287701, AJ287744, AJ287745	*Dendrosporobacter quericolum*	88.2–91.3	7 (4)	89.9–98.8
358ev, AJ287702	*Desulfosporosinus orientis*	93.3	1	
20ev, AJ287682-AJ287686	*Eubacterium contortum*	84.8–89.4	17 (6)	85.5–99.7
437ev, AJ287705	*Eubacterium desmolans*	82.4	2 (1)	100
430ev, AJ287710	*Eubacterium saburreum*	81.9	2 (1)	100
372ev, AJ287750	*Eubacterium* (unidentified)	–	1	
433ev, AJ287679, AJ287680	LB 313 uncultured Antarctic bacterium	82.1–82.8	3 (3)	86.9–95.7
444ev, AJ287688	*Ruminococcus hydrogenotrophicus*	84.5	2 (1)	100
411ev, AJ287693, AJ287694	*Sporomusa malonica*	87.6–89.4	11 (4)	94.6–99.4
75ev, AJ287697, AJ287740	*Sporomusa silvacetica*	89.0–90.6	2 (2)	92.7
102ev, AJ287689-AJ287691, AJ287741-AJ287743	*Sporomusa sphaeroides*	84.5–89.4	7 (4)	94.0–99.7
Actinobacteria				
8ev	*Acidimicrobium ferrooxidans*	77.9	1	
420ev	*Clavibacter michiganense*	79.5	1	
120ev, AJ287660	MC 87	86.1	1	
ARCHAEA				
a50ev	Ant12 Antarctic clone 371	97.9–98.6	6 (1)	99.3–100
a60ev	*Methanoculleus palmolei*	89.0–92.2	66 (2)	97.4–99.3
New group				
155ev	Unclassified clone	–	2 (1)	100

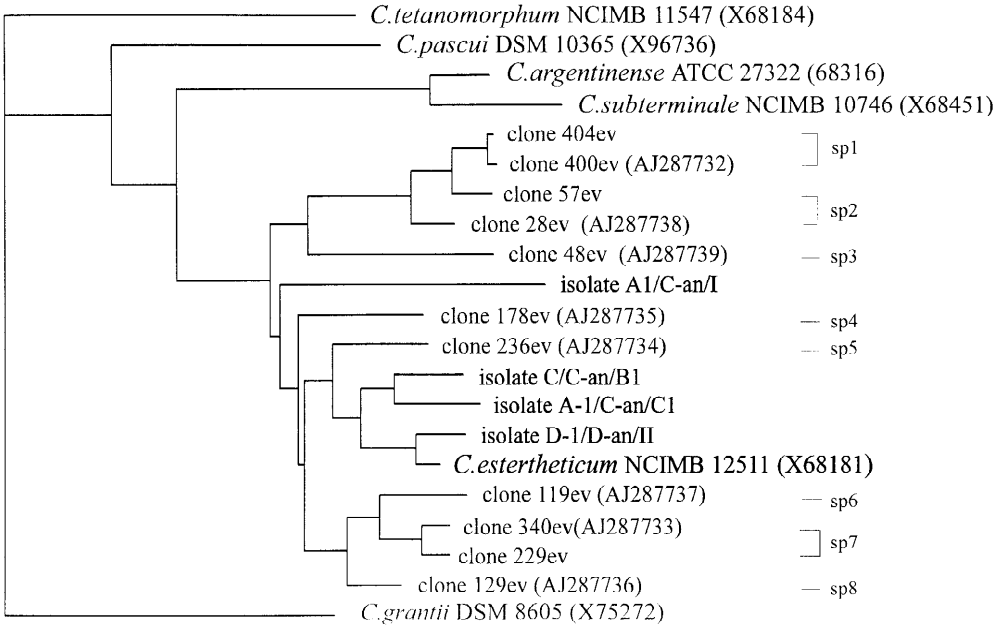

Fig. 1. Comparative analysis of partial 16S rDNA sequences (350 nucleotides from the 5′-terminus) of *Clostridium* species of cluster If (Stackebrandt and Hippe 2000), as well as isolates and some 16S rDNA clone sequences from Lake Fryxell shown to be related to *Clostridium estertheticum,* with the distance matrix analysis using the neighbor-joining method (De Soete 1983) and Jukes and Cantor correction (1969) to compensate for different evolutionary rates. *Bar* indicates 2% nucleotide substitutions; *sp*, potential species (see Table 3 ; *C. estertheticum*)

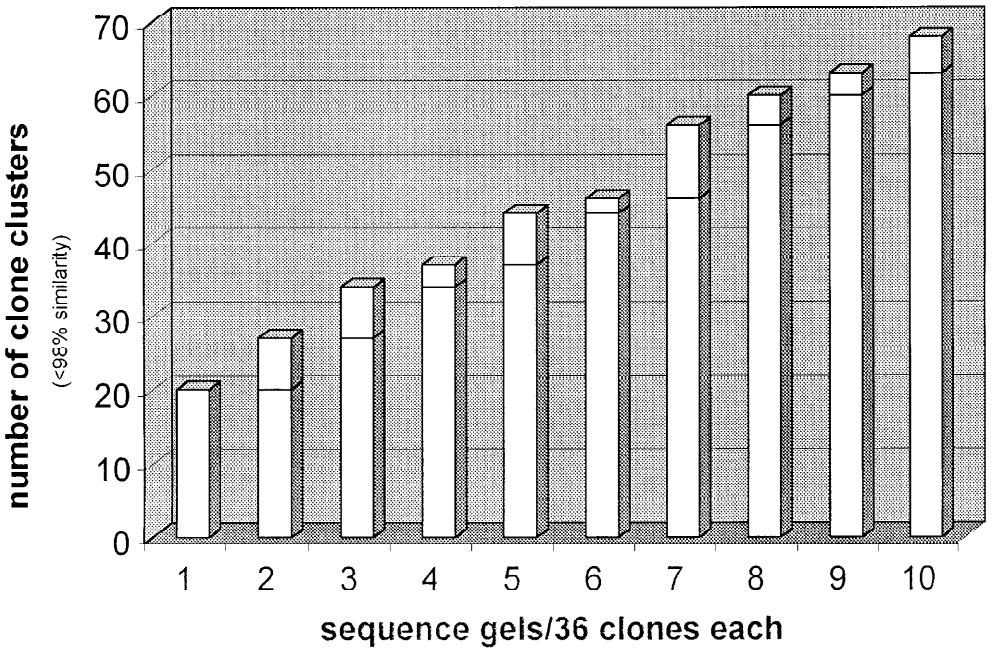

Fig. 2. Rarefaction curve showing the increase of higher taxonomic clusters in ten subsequently analyzed sequencing gels (36 sequences each). The *top part* of each column indicates the fraction of novel clusters as compared to the total number in the previously analyzed gel

gens are free-living and do not originate from a protozoa–intracellular methanogen association.

Discussion

The phylogenetic diversity of bacteria at both the species level and that of higher taxa may only be considered surprising if the Antarctic lakes are considered to be a virtually sterile environment. This assumption is certainly not true for moats that are formed in summer around the edges. Although Lake Fryxell itself is covered by perennial ice 3–4.5 m thick, the moat sites allow material to be transported into the lake from glaciers and land by streams and for airborne organisms to be carried by strong katabatic winds from as far afield as the South American continent. Sediment deposited on the ice surface migrates downward during the Antarctic summer, providing a constant input of inorganic and organic material, including microorganisms. In contrast to other Antarctic lakes, Lake Fryxell is old (10,000±9,000 years; Lyons et al. 1998), although there is evidence that Lake Fryxell became dry about 1,000–3,000 years ago and that the lake has refilled since (Chinn 1993; McKnight et al. 1998). The sampling site is close to the inflow of Huey Creek and Canada Stream, which contribute as much as 6% and 20%, respectively, of the annual stream flow into the lake, although these values fluctuate significantly depending on the year in which data have been generated (Conovitz et al. 1998). These streams not only discharge inorganic (PO_4^{3-} and NO_3^-) compounds but also a rich spectrum of organic material by the inflow of primary producers, such as algae and cyanobacteria (McKnight et al. 1998; Hawes and Howard-Williams 1998). On a volumetric basis, Fryxell is the most productive lake in the McMurdo Dry Valleys (James et al. 1998). The lake contains a complex protistan community (Laybourn-Parry et al. 1997), although no reports are available on prokaryotic diversity.

The large and diverse spectrum of microorganisms detected via 16S rDNA sequences and isolated cultures demonstrates the enormous complexity of the microbial community in the Antarctic lake mat. As already described for other studies comparing the 16S rDNA from prokaryotic cellular organisms and uncultured representatives, the match is low (Liesack and Stackebrandt 1992; Pukall et al. 1998; Rheims et al. 1998). A few taxa only were recovered by both strategies, here exemplified by the presence of sequences related to *Flavobacterium hibernum* and *Clostridium estertheticum*. The sequences of organisms that grow well under laboratory conditions, e.g., those related to *Arthrobacter flavus, Flavobacterium hibernum*, and *Janthinobacterium lividum*, are rarely, if at all, represented in clone libraries. If they are represented, their occurrence may be indicated by a single clone sequence only (i.e., *F. hibernum*).

It is interesting to note that some potential species belong to gliding bacteria (*Stigmatella, Myxococcus, Cytophaga, Flavobacterium, Marinilabilia,* and *Flexibacter*). Several psychrophilic and psychrotolerant cultured organisms that have been described recently from Antarctic environments, including sea ice, lake water and sediments, and soil and sandstone, are either members of the mat community or are at least represented by their 16S rDNA sequence. Examples are *Flavobacterium hibernum*, a gliding Gram-negative bacterium isolated from a freshwater

lake (McCammon et al. 1998); *Cryobacterium psychrophilum*, a soil actinobacterium (Suzuki et al. 1997); *Psychrobacter glacincola* (Bowman et al. 1997e), a halotolerant, lipolytic, and asaccharolytic bacterium from sea ice environments; *Carnobacterium alterfunditum* (Franzman et al. 1991), a saccharolytic, lactic acid-producing facultative anaerobe from anoxic waters in Ace Lake, Vestfold Hills; *Clostridium estertheticum* (Collins et al. 1992), a saccharolytic anaerobe originally isolated from vacuum-packed meat in the UK, but subsequently detected by sequence analysis of 16S rDNA clones in Lake Pendant; *C. vincentii* (Mountfort et al. 1998), a saccharolytic anaerobe from a low-salinity pond sediment of the McMurdo Ice Shelf; and *Arthrobacter flavus* (Reddy et al. 2000).

Although it is not possible to deduce the physiological properties of an organism from the phylogenetic position of its 16S rDNA when the similarities to its nearest cultured neighbor are distant, some general features of elements of the food web known to occur in mats can be observed. The sample appears to be rich in anaerobic fermenters, as exemplified by the presence of members of the *Clostridium* line of descent of Gram-positive organisms and members of *Bacteroides*. The community contains putative strains related to anaerobic saccharolytic organisms (e.g., *C. estertheticum, C. fallax, C. lentocellum, Eubacterium contortum,* and *Eubacterium saburreum*) forming CO_2, H_2, and C_1–C_4 acids and alcohols, which in turn are metabolized by other anaerobic members of the community (e.g., methanogens, acetogens [*Sporomusa*, *Acetonema*, *Acetobacterium*]), as well as by aerobes, facultative anaerobes, and phototrophic organisms. Polysaccharides may be derived from primary producers, described to be abundant in Lake Fryxell, and decomposers, e.g., *C. lentocellum, C. vinc*entii, and *C. termitidis*-like clostridia. Other bacteria are proteolytic (*C. limosum, C. subterminale*) or specialists, thriving on a restricted number of compounds only (e.g., *Pelobacter* sp.) The finding of some sequences related to those of sulfur compound-metabolizing organisms (*Desulfomonile* sp., *Desulfosporosinus*, and the aerobic *Bosea thiooxidans*) may indicate the presence of a sulfur cycle. The occurrence of aerobic organisms may be explained not only by the sedimentation effect but also by the sampling site, which is located in the shallow part of a moated zone; in this case the aerobic organisms are part of the metabolically active community in a structured mat, serving as a sink for gases, carbon, and other compounds as well as a nutrient source. The presence of ammonium oxidizers, reported to be abundant in the adjacent Lake Bonney (Voytek et al. 1998), and of other bacteria involved in the nitrogen cycle was not obvious from the 16S rDNA sequence information.

Comparison of the phylogenetic diversity of uncultured organisms of the Lake Fryxell mat sample with that of different sediments of hypersaline and marine-type salinity meromictic Antarctic lakes of the Vestfold Hills system (Bowman et al. 2000) reveals almost no similarity. Members of *Proteobacteria*, low G+C gram-positive bacteria, *Cytophagales*, and a few *Actinobacteria* were also found in the hypersaline lakes, but the phylogenetic affiliation of clones was different. Depending on the lake, the sediments also contained cyanobacteria/chloroplasts, including members of *Prochlorococcus*, σ-*Proteobacteria*, *Spirochaetales,* and *Chlamydiales,* as well as different euryarchaeal sequences. A large number of *Halobacteriales*-related clone sequences were present in Ekho Lakes. As discussed by Bowman (2000) for other Antarctic lakes, the presence of aerobic bacteria in anaerobic sediments, detected in the Lake Fryxell mat and in sediments of other Antarctic lakes, may be the result of their accumulation followed by slow decomposition of organic carbon. Comparison of the phylogenetic affiliation of the Lake Fryxell clone sequences to their nearest neighbors (Table 3 of this study) with those of clone sequences depicted in 16S rDNA dendrograms of Bowman et al. (2000) does not, except for two clones, indicate a close relationship.

A direct comparison of clone sequences generated in these studies is not possible because Bowman and coworkers based their analysis on a rDNA stretch ranging from position 522 to 1,500 whereas Lake Fryxell clones cover region positions 20 to 350. The only direct comparison can be made on the three *C. estertheticum* isolates, for which almost complete sequences have been generated, and clones PENDANT-1 (AF42915) and PENDANT-16 (AF14298). High similarity values, ranging from 98.6 to 99.3, indicate that strains related to the type strain of *C. estertheticum* thrive under different physiological conditions such as those found in Lake Pendant (2.3% salinity) and Lake Fryxell (freshwater). The diversity of clone sequences shown to be specifically related to *C. estertheticum* (Fig. 1) reveals a phenomenon that is commonly observed in 16S rDNA clone libraries of significantly different environmental samples (Pukall et al. 1998; Rheims and Stackebrandt 1998; Choi et al. 1994): a large number of clones are retrieved that possess only minor differences in their 16S rDNA. As demonstrated with *Alteromonas macleodii* clones (Pukall et al. 1998), some of the difference can be traced to operon microheterogeneity, but other differences might be interpreted as strain differences such as those also observed in the *C. estertheticum* isolates. This genomic diversity of phyloclusters may extend to other genes, which would allow a population of strains to react more flexibly to changes of physical and chemical conditions.

Acknowledgment This study was supported by a EU grant PL 970040 (MICROMAT). We thank Cathy Welch, who collected the mat material, and the Long Term Ecosystem Research Program (LTER), under whose auspices the material was collected.

References

Abyzov SS, Mitskevich IN, Poglazova MN (1998) Microflora of the deep glacier horizons of central Antarctica. Microbiology 67:451–458

Ashbolt NJ (1990) Antarctic biotechnology–what is the potential? Aust J Biotechnol 4:103–105

Bowman JP (1998) *Pseudoalteromonas prydzensis* sp. nov., a psychrotrophic, halotolerant bacterium from Antarctic sea ice. Int J Syst Bacteriol 43:1037–1041

Bowman JP, Cavanagh J, Austin J, Sanderson K (1996) Novel *Psychrobacter* species from Antarctic ornithogenic soils. Int J Syst Bacteriol 46:841–848

Bowman JP, McCammon SA, Brown JL, Nichols PD, McMeekin TA (1997a) *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria isolated from antarctic lacustrine and sea ice habitats. Int J Syst Bacteriol 47:670–677

Bowman JP, McCammon SA, Brown MV, Nichols DS McMeekin TA (1997b) Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl Environ Microbiol 63:3068–3078

Bowman JP, McCammon SA, Lewis T, Skerratt JH, Brown JL, Nichols DS, McMeekin TA (1998) *Psychroflexus torquis* gen. nov., sp. nov., a psychrophilic species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al. 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. Microbiology 144:1601–1609

Bowman JP, McCammon SA, Nichols DS, Skerratt JH, Rea SM, Nicols PD, McMeekin TA (1997c) S*hewanella gelidimarina* sp. nov. and *Shewanella frigidimarina* sp. nov., novel Antarctic species with the ability to produce eicosapentaenoic acid (20:5ω3) and grow anaerobically by dissimilatory Fe(III) reduction. Int J Syst Bacteriol 47:1040–1047

Bowman JP, McCammon SA, Skerratt JH (1997d) *Methylosphaera hansonii* gen. nov., sp. nov., a psychrophilic, group I methanotroph from Antarctic marine-salinity, meromictic lakes. Microbiology 143:1451–1459

Bowman, JP, Nichols DS, McMeekin TA (1997e) *Psychrobacter glacincola* sp. nov., a halotolerant, psychrophilic bacterium isolated from Antarctic sea ice. Syst Appl Microbiol 20:209–215

Bowman JP, Rea SM, McCammon SA, McMeekin TA (2000) Diversity and community structure within anoxic sediment from marine salinity meromictic lakes and a coastal meromictic marine basin, Vestfold Hills, Eastern Antarctica. Environ Microbiol 2:227–237

Bozal N, Tudela E, Roselló-Mora R, Lalucat J Guinea J (1997) *Pseudoalteromonas antarctica* sp. nov., isolated from an Antarctic coastal environment. Int J Syst Bacteriol 47:345–351

Brosius JM, Palmer L, Kennedy P, Noller HF (1978) Complete nucleotide sequence of the 16S ribosomal RNA gene from *Escherichia coli*. Proc Natl Acad Sci USA 75:4801–4805

Cambell R, Greaves MP (1990) Methods for studying the microbial ecology of the rhizosphere. Methods Microbiol 22:447–477

Chinn TH (1993) Physical hydrology of the Dry Valley Lakes. In: Green WJ, Friedman EI (eds) Physical and biogeochemical processes in Antarctic lakes. Antarctic research series 59. American Geophysical Union, Washington, DC, pp 1–52

Choi B-K, Paster BJ, Dewhirst FE, Göbel UB (1994) Diversity of cultivable and uncultivable oral spirochetes from a patient with severe destructive periodontitis. Infect Immun 62:1889–1895

Collins MD, Rodrigues UM, Dainty RH, Edwards RA, Roberts TA (1992) Taxonomic studies on a psychrophilic *Clostridium* from vacuum-packed beef: description of *Clostridium estertheticum* sp. nov. FEMS Microbiol Lett 96:235–240

Conovitz PA, McKnight D, MacDonald LH, Fountain AGM, House HR (1998) Hydrologic processes influencing streamflow variation in Fryxell Basin, Antarctica. In: Priscu JC (ed) Ecosystem dynamics in a polar desert. The McMurdo Dry Valleys, Antarctica. Antarctic research series 72. American Geophysical Union, Washington, DC, pp 93–108

DeSoete G (1983) A least squares algorithm for fitting additive trees to proximity data. Psychometrika 48:621–626

Dobson SJ, Colwell RR, McMeekin TA, Franzmann PD (1993) Direct sequencing of the polymerase chain reaction-amplified 16S rRNA gene of *Flavobacterium gondwanense* sp. nov. and *Flavobacterium salegens* sp. nov., two new species from a hypersaline Antarctic lake. Int J Syst Bacteriol 43:77–83

DSMZ (1998) Catalogue of strains, 6th edn. Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig

Franzmann PD, Höpfl P, Weiss N, Tindall BJ (1991) Psychrotrophic, lactic acid-producing bacteria from anoxic waters in Ace Lake, Antarctica; *Carnobacterium funditum* sp. nov. and *Carnobacterium alterfunditum* sp. nov. Arch Microbiol 156:255–262

Franzmann PD, Liu Y, Balkwill DL, Aldrich HC, Conway de Macario E, Boone DR (1997) *Methanogenium frigidum* sp. nov., a psychrophilic, H_2-using methanogen from Ace Lake, Antarctica. Int J Syst Bacteriol 47:1068–1072

Friedmann EI (1980) Endolithic microbial life in hot and cold deserts. Origins Life 10:223–235

Gosink JJ, Staley JT (1995) Biodiversity of gas vacuolate bacteria from Antarctic sea ice and water. Appl Environ Microbiol 61:3486–3489

Gosink JJ, Woese CR, Staley JT (1998) *Polaribacter* gen. nov., with three new species, *P. irgensii* sp. nov., *P. franzmannii* sp. nov. and *P. filamentus* sp. nov., gas vacuolate polar marine bacteria of the *Cytophaga-Flavobacterium-Bacteroides* group and reclassification of *'Flectobacillus glomeratus'* as *Polaribacter glomeratus* comb. nov. Int J Syst Bacteriol 48:223–235

Griepenburg U, Ward-Rainey N, Mohamed S, Schlesner H, Marxsen H, Rainey FA, Stackebrandt E, Auling G (1996) Phylogenetic diversity, polyamine pattern and DNA base composition of members of the order *Planctomycetales*. Int J Syst Bacteriol 49:689–696

Hawes I, Howard-Williams C (1998) Primary production processes in streams of the McMurdo Dry Valleys, Antarctica. In: Priscu JC (ed) Ecosystem dynamics in a polar desert. The McMurdo Dry Valleys, Antarctica. Antarctic research series 72. American Geophysical Union, Washington, DC, pp 129–140

Hirsch P, Ludwig W, Hethke C, Sittig M, Hoffmann B, Gallikowski CA (1998) *Hymenobacter roseosalivarius* gen. nov., sp. nov. from continental Antartica soils and sandstone: bacteria of the *Cytophaga/Flavobacterium/Bacteroides* line of phylogenetic descent. Syst Appl Microbiol 21:374–383

Irgens RL, Gosink JJ, Staley JT (1996) *Polaromonas vacuolata* gen. nov., sp. nov., a psychrophilic, marine, gas vacuolate bacterium from Antarctica. Int J Syst Bacteriol 46:822–826

James MR, Hall JA, Laybourn-Parry J (1998) Protozooplankton and microzooplankton ecology in lakes of the Dry Valleys, Southern Victoria Land. In: Priscu JC (ed) Ecosystem dynamics in a polar desert. The McMurdo Dry Valleys, Antarctica. Antarctic research series 72. American Geophysical Union, Washington, DC, pp 255–267

Jay JM (1979) Comparison of homogenizing, shaking and blending on the recovery of microorganisms and endotoxins from fresh and frozen ground beef. Appl Environ Microbiol 38:879–884

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism. Academic Press, New York, pp 21–132

Junge K, Gosink JJ, Hoppe H-G, Staley JT (1998) *Arthrobacter, Brachybacterium* and *Planococcus* isolates identified from antarctic sea ice brine. Description of *Planococcus mcmeekinii*, sp. nov. Syst Appl Microbiol 21:306–314

Kriss AE (1976) Microbiological studies of the Wanda Lake. Mikrobiologia 45:1075–1081

Labrenz M, Collins MD, Lawson PA, Tindall BJ, Braker G, Hirsch P (1998) *Antarctobacter heliothermus* gen. nov., sp. nov., a budding bacterium from hypersaline and heliothermal Ekho Lake. Int J Syst Bacteriol 48:1363–1372

Laybourn-Parry J, James MR, McKnight D, Priscu J, Spaulding S, Shiel R (1997) The microbial plankton of Lake Fryxell, Southern Victoria land, Antarctica. Polar Biol 17:54–61

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

Lyons WB, Welch KA, Neumann K, Toxey JK, McArthur R, Williams C, McKnight DM, Moorhead D (1998) Geochemical linkages among glaciers, streams and lakes within the Taylor Valley, Antarctica. In: Priscu JC (ed) Ecosystem dynamics in a polar desert. The McMurdo Dry Valleys, Antarctica. Antarctic research series 72. American Geophysical Union, Washington, DC, pp 77–92

Marshall BJ (1966) *Bacillus macquariensis* n. sp., a psychrotrophic bacterium from sub-antarctic soil. J Gen Microbiol 44:41–46

Maugeri TL, Gugliandolo C, Bruni V (1996) Heterotrophic bacteria in the Ross Sea (Terra Nova Bay, Antarctica). Microbiologica 19:67–76

McCammon SA, Innes BH, Bowman J, Franzmann PD, Dobson SJ, Holloway PE, Skeratt JH, Nichols PD, Rankin LM (1998) *Flavobacterium hibernum* sp. nov., a lactose-utilizing bacterium from a freshwater Antarctic lake. Int J Syst Bacteriol 48:1405–1412

McKnight D, Alger A, Tate CM, Shupe G, Spaulding S (1998) Longitudinal patterns in algal abundance and species distribution in melt-

water streams in Taylor Valley, Southern Victoria Land, Antarctica. In: Priscu JC (ed) Ecosystem dynamics in a polar desert. The McMurdo Dry Valleys, Antarctica. Antarctic research series 72. American Geophysical Union, Washington, DC, pp 109–127

Mountfort DO, Rainey FA, Burghardt J, Kaspar HF, Stackebrandt E (1998) *Clostridium vincentii* sp. nov., a new obligately anaerobic, saccharolytic, psychrophilic bacterium isolated from low-salinity pond sediment of the McMurdo Ice Shelf, Antarctica. Arch Microbiol 167:54–60

Murray AE (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl Environ Microbiol 64:2585–2595

Osnitskaia LK (1978) Photosynthesizing bacteria from the Wanda Lake. Mikrobiologia 47:131–137

Pfister RM (1965) Numerical taxonomy of some bacteria isolated from antarctic and tropical seawaters. J Bacteriol 90:863–872

Priscu JC (1998) Perennial Antarctic lake ice: an oasis for life in a polar desert. Science 280:2095–2098

Pukall R, Päuker O, Buntefuß D, Ulrichs G, Lebaron P, Bernhard L, Guindulain T, Vives-Rego J, Stackebrandt E (1998) High sequence diversity of *Alteromonas macleodii*-related cloned and cellular 16S rDNAs from a Mediterranean seawater mesocosm experiment. FEMS Microb Ecol 28:335–344

Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E (1996) The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. Int J Syst Bacteriol 46:1088–1092

Reddy GSN, Aggarwal RK, Matsumoto GI, Shivaji S (2000) *Arthrobacter flavus* sp. nov., a psychrophilic bacterium isolated from a pond in McMurdo Dry Valley, Antarctica. Int J Syst Evol Microbiol 50:1553–1561

Rheims H, Stackebrandt E (1998) Application of nested PCR for the detection of as yet uncultured organisms of the class Actinobacteria in environmental samples. Mol Ecol 1:137–143

Rheims H, Felske A, Seufert S, Stackebrandt E (1998) Molecular monitoring of an uncultured group of the class Actinobacteria in two terrestrial environments. J Microbiol Methods 36:65–75

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Schumann P, Prauser H, Rainey FA, Stackebrandt E, Hirsch P (1997) *Friedmanniella antarctica* gen. nov., sp. nov., an LL-diaminopimelic acid-containing actinomycete from Antarctic sandstone. Int J Syst Bacteriol 47:278–283

Stackebrandt E, Goebel BM (1994) A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Stackebrandt E, Hippe H (2000) Taxonomy and systematics. In: Bahl H, Dürre P (eds) Clostridia: biotechnology and medical applications. Wiley-VCH, Weinheim (in press)

Stackebrandt E, Rainey FA (1995) Partial and complete 16S rDNA sequences, their use in generation of 16S rDNA phylogenetic trees and their implications in molecular ecological studies. In: Akkermans ADL, van Elsas JD, de Bruijn FJ (eds) Molecular microbial ecology manual, vol 3.1.1. Kluwer, Dordrecht, pp 1–17

Suzuki K-I, Sasaki J, Uramoto M, Nakase T, Komagata K (1997) *Cryobacterium psychrophilum* gen. nov., sp. nov., nom. rev., comb. nov., an obligately psychrophilic actinomycete to accommodate "*Curtobacterium psychrophilum*" Inoue and Komagata 1976. Int J Syst Bacteriol 47:474–447

Tindall BJ, Brambilla E, Steffen M, Neumann R, Pukall R, Kroppenstedt RM, Stackebrandt E (2000) Cultivatable microbial diversity: gnawing at the Gordian knot. Environ Microbiol 2:310–318

Tsyganov VA (1970) Detection and morphological-cultural characteristics of actinomyces from the Antarctic. Mikrobiologia 39:821–826

von Wintzingerode F, Göbel U, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 21:213–229

Voytek MA, Ward BB, Pricu JC (1998) The abundance of ammonium-oxidizing bacteria in Lake Bonney, Antarctica, determined by immunofluorescence, PCR, and in situ hybridization. In: Priscu JC (ed) Ecosystem dynamics in a polar desert. The McMurdo Dry Valleys, Antarctica. Antarctic research series 72. American Geophysical Union, Washington, DC, pp 217–228