Microbial Ecology

Bacterial Diversity in Three Different Antarctic Cold Desert Mineral Soils

Jacques J. Smith[1], Lemese Ah Tow[1], William Stafford[1], Craig Cary[2,3] and Donald A. Cowan[1]

(1) Department of Biotechnology, University of the Western Cape, Bellville 7535, Cape Town, South Africa
(2) Department of Biological Sciences, University of Waikato, Hamilton, New Zealand
(3) College of Marine Studies, University of Delaware, Lewes, Delaware, USA

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Abstract

A bacterial phylogenetic survey of three environmentally distinct Antarctic Dry Valley soil biotopes showed a high proportion of so-called "uncultured" phylotypes, with a relatively low diversity of identifiable phylotypes. Cyanobacterial phylotypic signals were restricted to the high-altitude sample, whereas many of the identifiable phylotypes, such as the members of the Actinobacteria, were found at all sample sites. Although the presence of Cyanobacteria and Actinobacteria is consistent with previous culture-dependent studies of microbial diversity in Antarctic Dry Valley mineral soils, many phylotypes identified by 16S rDNA analysis were of groups that have not hitherto been cultured from Antarctic soils. The general belief that such "extreme" environments harbor a relatively low species diversity was supported by the calculation of diversity indices. The detection of a substantial number of uncultured bacterial phylotypes showing low BLAST identities (<95%) suggests that Antarctic Dry Valley mineral soils harbor a pool of novel psychrotrophic taxa.

Introduction

Temperate and tropical soil communities are regarded as among the most complex and diverse assemblages of microorganisms [27], with estimated bacterial numbers in the order of 10^9 cells g^{-1} and over 10^4 distinct genospecies, as shown by reassociation kinetics [15, 47]. However, the desiccated mineral soils of the Dry Valleys, Ross Dependency, Eastern Antarctica, are generally thought to harbor very low cell densities [6–8], supporting the perception that the so-called extreme environments exhibit low species diversity and low cell numbers. This is attributed to the imposition of environmental extremes [37], which, for the Antarctic Dry Valleys, include low temperatures, wide temperature fluctuations, low nutrient status, low water availability, high incident radiation, and physical disturbance [54]. Nevertheless, we have recently shown by ATP, lipid, and DNA quantitation that Dry Valley mineral gravels may contain between 10^6 and 10^8 prokaryotic cells g^{-1} [12].

The current understanding of Antarctic mineral soil microbiology is based almost exclusively on culture-based studies. These studies have suggested that most Antarctic microbes belong to a restricted number of cosmopolitan taxa and are largely aerobic, with only few reported anaerobic isolates [19]. Large numbers of coryneform-related bacteria such as *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, and *Corynebacterium* were reported together with gracilicutean isolates (members of the Gram-negative Eubacteria) such as *Pseudomonas* and *Flavobacterium*. Firmicutean bacteria isolated included *Bacillus*, *Micrococcus*, *Nocardia*, *Streptomyces*, *Flavobacterium*, and pseudomonads [6, 19]. A number of less common genera such as *Beijerinckia*, which rarely occur outside tropical soils, *Xanthomonas*, a pathogen associated with higher plants, and *Planococcus*, a marine genus, have also been isolated from Antarctic soils [19]. Cyanobacteria are also well-documented inhabitants of Antarctic soil biotopes [13, 46] but are thought to be restricted to moist habitats [14].

It is now widely acknowledged that culture-based community studies inevitably induce a high degree of bias, whereas important groups of organisms that may be fastidious, co-culture-dependent or in a viable but nonculturable (VBNC) state may be unrepresented [2, 21, 31, 53]. It is therefore probable that historical data from culture-dependent studies do not accurately represent the true microbial species diversity of the Dry Valley mineral soils.

Correspondence to: Donald A. Cowan; E-mail: dcowan@uwc.ac.za

A number of important Antarctic Dry Valley microbial biotopes, including cryptoendolithic communities [13], cryoconite holes [10], and lake ice and marine ice flows [40, 43], have been subject to detailed community analyses using modern molecular phylogenetic techniques. However, the supposedly less complex and more "extreme" mineral soils have yet to be investigated in detail. Here we present "snapshot" phylogenetic analyses of the bacterial diversity in three environmentally distinct Ross Dependency mineral soil biotopes.

Materials and Methods

Sample Sites. Mineral soil samples were collected from three dry mineral soil sites: (i) underneath a crabeater seal carcass on Bratina Island (BIS1), (ii) the midslopes of Miers Valley (MVG), and (iii) fine gravels from Penance Pass, a high-altitude site between the Miers and Shangri La Valleys (PENP). Physical and chemical characteristics such as lipid, water, and protein content of each site was determined and are listed in Table 1. Samples were recovered under aseptic conditions by removal of a 1-cm surface layer of mineral soil from a 20 × 20-cm sample area. All samples (approx. 400 g) were mixed thoroughly and resampled before storage at <0°C for transport. Samples were stored at −80°C in the laboratory.

Lipid Analysis. Total lipid was extracted according to the method of Folch [18]. Twenty milliliters of chloroform/methanol (2:1 v/v) was added to 0.5 g of each soil sample. Samples were agitated for 15 min at room temperature prior to centrifugation and aspiration of the top phase. Methanol/water (1:1 v/v) was used to wash the interface without disturbing the lipid-containing lower phase. Samples were recentrifuged, the lower phase recovered, methanol aspirated, and the chloroform allowed to evaporate. The gel-like lipid fraction was stored at −20°C until required. Lipid content was determined gravimetrically.

Total Protein and Water Content Determination. Total protein was determined using the Bio-Rad protein assay (Hercules, CA, USA). Samples of soil (0.5 g) were added to a modified lysis buffer (equal volumes of 100 mM NaH_2PO_4, 100 mM NaCl, 500 mM Tris, pH 8.0, 10% sodium dodecyl sulfate) as described [33]. The vials were placed in a FastPrep Bead-Beater (BIO 101, Vista, CA, USA) at 4.5 ms for 40 s, after which they were centrifuged and 200 μL of the supernatant was used in the microassay procedure as outlined by the manufacturer. Optical density measurements were performed at OD_{595} and plotted against a 1- to 20-μg bovine serum albumin standard curve.

Water content was determined by placing at least 10 g of soil in a preweighed and dried glass petri dish. Samples, in triplicate, were incubated to constant weight at 100°C over a period of 72 h. Soil and water content was calculated as a percentage of initial weight.

DNA Extraction and 16S rRNA Gene Polymerase Chain Reaction Amplification. Bulk genomic DNA was extracted from 0.5 g of mixed soil from each site using the FastDNA® spin kit for soil (BIO 101). The universal eubacterial 16S rDNA primers E9F (5′-GAG TTT GAT CCT GGC TCA G-3′) [17] and U1510R (5′-GGT TAC CTT GTT ACG ACT T-3′) [41] were used to amplify a 1.5-kb region of the 16S rRNA genes. A typical polymerase chain reaction (PCR) reaction contained (final concentration) 1× PCR buffer, 1.5 mM $MgCl_2$, 0.5 μL recombinant *Taq* polymerase, 0.5 μM (each) primer, 200 μM of each deoxynucleoside triphosphate, and approximately 10–15 ng template DNA in a 50-μL reaction volume. PCR conditions were as follows: 96°C for 2 min; 30 cycles of 96°C for 1 min, 50°C for 1 min, 72°C for 1 min; and a final incubation at 72°C for 10 min. PCR products were electrophoresed and visualized on a 1% agarose gel and subsequently purified with the GFX PCR DNA and gel band purification kit (Amersham Biosciences, Piscataway, NJ, USA).

Denaturing Gradient Gel Electrophoresis. PCR with 16S rDNA denaturing gradient gel electrophoresis (DGGE) primers 341fgc and 534r was carried out as previously described [52]. PCR amplicons (2–20 μL equivalent to 100–500 ng DNA for metagenomic samples) were separated by DGGE [35] on 16.5 × 16.5-cm, 1-mm-

Table 1. Site descriptions for Dry Valley mineral soil samples

Sample code	*Site description*	*GPS*	*DNA (ng/g)*	*Protein (mg/g)*	*Lipid (μg/g)*	*Altitude (m)*	*(H_2O) (% wt.)*
PENP	Dry sand from high-altitude site	78° 04.762′, 165° 52.083′	480	280	86	584	0.7
MVG	Dry sorted sands and gravels from mid-altitude valley slopes	78° 06.140′, 165° 48.646′	840	386	66	225	0.8
BIS	Fine, dark, particulate the soil from underside of seal carcass	78° 00.966′, 165° 32.795	320	656	326	2	6.2

thick 9% polyacrylamide [37.5:1 (w/v) acrylamide/bis-acrylamide, Fluka, Ronkonkema, NY, USA] gels with 40–60% UF gradients [100% UF = 7 M urea, 40% (v/v) formamide], using SciPlas (V20-HCDC) apparatus. Electrophoresis was carried out at 100 V for 16 h at 60°C in 1× Tris–acetate–EDTA (TAE) buffer. Gels were stained (0.5 μg/mL ethidium bromide in 1× TAE for 10 min), destained (1× TAE for 15 min), and visualized with a 312-nm wavelength transilluminator (Spectroline, Westbury, NY, USA).

16S rDNA Clone Libraries, Amplified rDNA Restriction Analysis, and Sequencing. Purified 16S rDNA amplicons were cloned into the pMOS*Blue* vector using the pMOS*Blue* blunt-ended cloning kit (Amersham Pharmacia Biotech). Recombinant plasmids were randomly chosen for colony PCR with M13F/M13R vector-derived primers. Amplified rDNA restriction analysis (ARDRA) [30] was performed on selected PCR products. The amplicons were digested with *Eco*RI and *Afa*I and electrophoresed on 1% and 2% agarose gels, respectively. Restriction digestion profiles were compared to avoid sequencing redundant clones. Clones containing recombinant plasmids showing unique ARDRA patterns were grown overnight in Luria–Bertani medium supplemented with ampicillin (100 μg mL^{-1}) at 37°C. Plasmid DNA was extracted using the GFX Micro plasmid prep kit (Amersham Biosciences) for sequencing with the MegaBACE 500 Automated Capillary DNA Sequencing System (Amersham Biosciences). The primer used for sequencing was an internal 16S rDNA primer: F3:S16.5 (nt 517–536 on *Escherichia coli* 16S rDNA sequence) (5′-GCC AGC AGC CGC GGT AAT AC-3′).

Phylogenetic Analysis. Unique rDNA sequences of ≥500 bp were compared with current database sequences (GenBank) using NCBI's Basic Local Alignment Search Tool (BLAST) to determine phylogenetic relatedness [1]. Chimeric sequences were identified using CHECK_CHIMERA [29]. Coverage analysis of the sample sites was performed according to Dunbar *et al.* [16].

Nucleotide Accession Numbers. The nucleotide sequences determined in this study have been deposited in the NCBI database under accession nos. DQ062859 to DQ062918.

Results

Sampling Homogeneity. To address the question of whether the sampling strategy provided representative data on microbial diversity, we used DGGE to assess local homogeneity. Mineral soil samples were recovered from the MVG site (225 m altitude) and a second midslope site at 384 m altitude using a strategy designed to assess microheterogeneity. At each altitude, three sites were selected across a 50-m horizontal transect (at 0, 25, and 50 m). At each site, four samples of surface (0–2 cm) mineral soil were collected aseptically from the quadrants of a 1-m^2 quadrant. The four samples were mixed and resampled. DGGE patterns from amplification of DNA extracts (Fig. 1) show a high level of local species homogeneity, at least within this altitudinal range.

Soil Nutrients. The three mineral soils chosen for comparison differ significantly in environmental aspect and protein, lipid, and water status (Table 1). Sample BIS is considered to represent a less extreme biotope than the other samples. This site benefits from relatively high water content (6.2%), some 8-fold higher than the other two sites, and from carbon supplementation provided by the immediate presence of a crabeater seal carcass [51]. The local seeding of the mineral soils by lipid-rich cellular material from the decomposing/fragmenting seal carcass

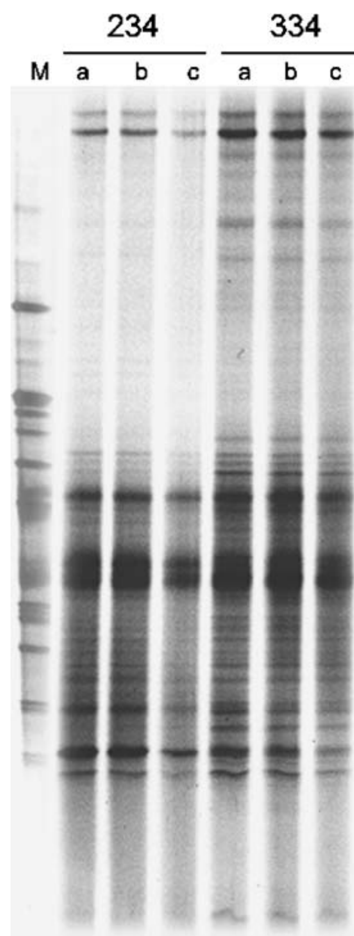

Figure 1. DGGE profiles of 16S rRNA gene amplicons from Miers Valley mineral soil samples. Samples were recovered from 284 and 384 m altitude and from three 1-m^2 quadrants across a 50-m horizontal transect at 0 (a), 25 (b) and 50 m (c). M is a molecular weight marker.

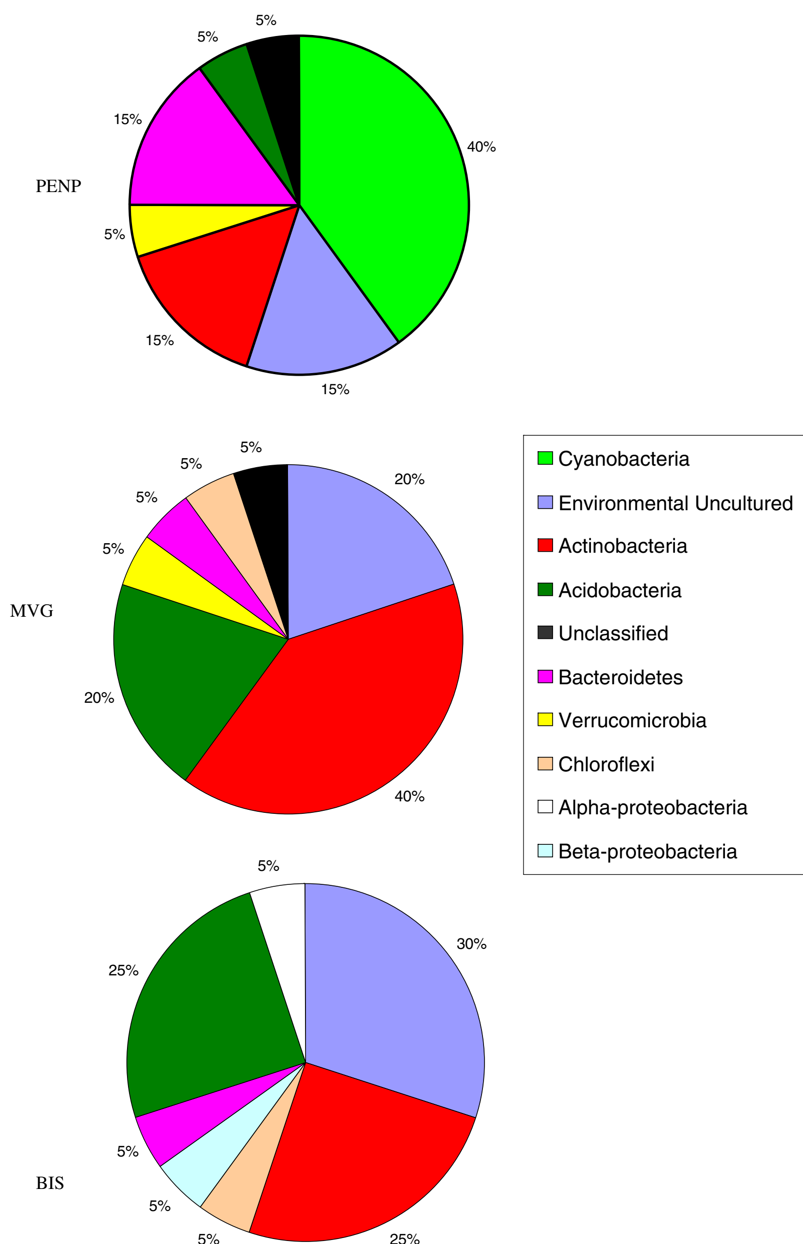

Figure 2. Relative percentage abundances of the principal phylotypic groups found in three distinct mineral soil sample sites in the Ross Dependency, Antarctica.

probably accounts for the high titer of extractable lipid (326 μg/g soil).

Clone Library Selection and Screening. 16S rDNA libraries were prepared from total community DNA isolated from three separate locations within the Ross Dependency region (Table 1). In total, 181 clones containing inserts of the correct size were subjected to ARDRA analysis for broad classification. Of the BIS clones, 57% showed unique ARDRA patterns, compared with 51% for MVG and 43% for PENP. To test the reliability of the ARDRA analysis, a series of clones showing identical ARDRA patterns were sequenced. All clones from unique ARDRA clades showed identical sequences.

Coverage curves (data not shown) indicated that all three samples were tending toward phylotypic saturation, but that the more eutrophic BIS sample was approaching the diversity plateau rather more slowly than the oligotrophic samples. The loss of linearity after analysis of a few tens of independent clones is strongly suggestive that these biotopes have relatively low species diversity, or are dominated by a relatively low number of phylotypes. Diversity, as determined by calculation of the coverage index [46] indicated the following: MVG 64%, PENP 73%, BIS 56%.

16S rDNA Sequence Analyses. Partial 16S rRNA sequences were obtained using the internal primer

Table 2. 16S rDNA clones identified in three different mineral soil sample sites

Sample site/clones	*Phylogenetic group*	*I.D. of nearest match (accession no.)*	*% ID*
PENP4	Cyanobacteria	*Leptolyngbya* sp. (AY239604)	96
PENP7	Actinobacteria	*Arthrobacter agilis* (AF134184)	99
PENP18	Cyanobacteria	*Phormidium* sp. Ant-Lunch (AF263335)	99
PENP22	Cyanobacteria	Uncultured Antarctic bacterium (AF076163)	97
PENP25	Cyanobacteria	Uncultured Antarctic cyanobacterium (AY151721)	95
PENP35	Environmental samples	Uncultured organism clone (AY897885)	93
PENP37	Bacteroidetes	Uncultured Bacteroidetes (AY689627)	97
PENP40	Actinobacteria	Uncultured Actinobacterium clone FBP460 (AY250884)	99
PENP42	Bacteroidetes	Uncultured bacterial clone (AJ290025)	96
PENP48	Verrucomicrobia	Uncultured soil bacterial clone C019 (AF013522)	95
PENP49	Cyanobacteria	*Nostoc* sp. (AY566855)	98
PENP50	Bacteria, environmental sample	Uncultured bacterial clone csbio160368 (AY187335)	98
PENP54	Actinobacteria	Uncultured Actinobacterium (AY690206)	92
PENP55	Bacteroidetes	Uncultured Bacteroidetes cloneVC5 (AY211071)	96
PENP62	Acidobacteria	Uncultured *Acidobacterium* (AY571794)	92
PENP68	Bacteria, environmental samples	Uncultured bacterium (AY662047)	93
PENP75	Bacteria, environmental sample	Uncultured bacterial clone sipK9 (AJ307936)	93
PENP76	Cyanobacteria	*Oscillatoria* sp. Ant-G16 (AF26333)	99
PENP77	Cyanobacteria	*Trichormus azollae* (AJ630454)	95
PENP78	Cyanobacteria	Uncultured Antarctic cyanobacterium (AY151722)	97
MVG1	Actinobacteria	*Geodermatophilus* sp. G1S (X92364)	94
MVG2	Bacteria, environmental sample	Uncultured bacterial clone C-F-15 (AF443586)	96
MVG5	Actinobacteria	Uncultured Actinobacterium (AY690226)	94
MVG9	Verrucomicrobia	Uncultured bacterial clone CO19 (AF013522)	96
MVG14	Actinobacteria	Bacterium Ellin504 (AY960767)	95
MVG15	Actinobacteria	*Conexibacter woesei* (AJ440237)	96
MVG16	Acidobacteria	Uncultured *Acidobacterium* (AY571792)	98
MVG18	Bacteria, environmental sample	Uncultured bacterial clone FBP241 (AY250867)	95
MVG19	Bacteria, environmental sample	Uncultured bacterial clone SO27 (AF013554)	96
MVG20	Bacteria, environmental sample	Uncultured bacterial clone D132 (AY274138)	95
MVG21	Acidobacteria,	Uncultured *Acidobacterium* (AY571794)	94
MVG23	Actinobacteria	Uncultured Actinobacterium (AF234135)	94
MVG24	Chloroflexi	Uncultured Chloroflexi bacterium (AY922044)	98
MVG25	Acidobacteria	Uncultured acidobacterial clone BAC-220H (AY214900)	92
MVG28	Bacteriodetes	Uncultured Bacteroidetes (AY921683)	96
MVG31	Actinobacteria	*Actinobacteria* strain PB90-4 (AJ229240)	96
MVG50	Actinobacteria	*Kribella* sp. (AJ811962)	95
MVG51	Actinobacteria	Bacterium Ellin301 (AF498683)	95
MVG52	Unclassified, environmental sample	Uncultured organism (AY897921)	94
MVG54	Bacteria, environmental samples	Uncultured bacteria candidate division OP10 (AY192276)	92
BIS1	Actinobacteria	*Intrasporangium calvum* DSM 43043T (AJ566282)	97
BIS2	Actinobacteria	Uncultured Actinobacterium (AY250884)	96
BIS5	Bacteria, environmental samples	Uncultured soil bacterial clone CO133 (AF507702)	91
BIS6	Bacteroidetes	Uncultured *Flavobacterium* sp. (AY571822)	96
BIS8	β-proteobacteria	Uncultured β-proteobacterium (AY690290)	95
BIS10	α-proteobacteria	Uncultured α-proteobacterium (AJ532707)	95
BIS12	Acidobacteria	Uncultured *Acidobacterium* (AY571792)	98
BIS13	Actinobacteria	Uncultured Actinobacterium (AF498707)	96
BIS15	Acidobacteria	Uncultured *Acidobacterium* (AY281358)	95
BIS16	Acidobacterium	Uncultured *Acidobacterium* (AY921997)	92
BIS18	Acidobacteria	Uncultured *Acidobacterium* (AY921984)	91
BIS20	Bacteria, environmental sample	Agricultural soil bacterial clone SC-I-54 (AJ252640)	93

Table 2. Continued

Sample site/clones	*Phylogenetic group*	*I.D. of nearest match (accession no.)*	*% ID*
BIS21	Bacteria Environmental samples	Uncultured bacterium (AJ1863268)	98
BIS23	Bacteria Environmental samples	Uncultured bacterial clone D130 (AY274136)	95
BIS25	Acidobacteria	Uncultured *Acidobacterium* (AY214900)	91
BIS26	Bacteria, environmental samples	Uncultured soil bacterium (AY493980)	91
BIS28	Actinobacteria	Uncultured *Rubrobacter* sp. (AY571811)	98
BIS29	Actinobacteria	Uncultured Rubrobacterium (AY395449)	91
BIS31	Bacteria, candidate division OP10	Uncultured bacterial clone W1-4H (AY192276)	92
BIS45	*Chloroflexus*	Uncultured bacterium #0319-23B10 (AF234122)	91

F3:S16.5, which includes the complete V5 and V6 variable regions and part of the V4 region. The program CHECK_CHIMERA identified two chimeric sequences that were subsequently omitted. To ensure that the largest possible number of nucleotides were included in the BLAST analysis, sequences yielding ≥500 bp were compared against all available 16S rDNA phylotypes in the NCBI ribosomal database [1]. Only eight broad phylotypic groups (Cyanobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia, α-Proteobacteria, β-Proteobacteria, Chloroflexi, and Bacteroidetes) were identified from the total of 61 sequences representing all 181 clones (Table 2). Three phylotypic groups showed distribution in all three sites where the remaining five were either unique to one or two sites.

The phylotypic groups showed an average identity of ≥91% to known phylotypes in GenBank. Overall, Cyanobacteria (13%), Actinobacteria (26%), and Acidobacteria (16%) represented the majority of the identified phylotypes. Cyanobacteria appeared to be restricted to only the high-altitude PENP sample site. Actinobacterial signals were frequent in all three sites, a result that is consistent with the distribution of actinobacteria [4]. The acidobacterial signals were the most highly populated clade in the nutrient-rich BIS sample (Fig. 2). Eighteen percent of all phylotypic signals obtained were assigned as so-called “uncultured” and were prevalent in all three sites (Fig. 2).

Most prominent within the PENP clone set were two orders of cyanobacteria; *Oscillatoriales* and *Nostocales*. Clone PENP49 showed close relatedness (98%) to *Peltigera pruinosa* cyanobiont 18 and PENP18 to *Phormidium* sp. Ant-lunch (99%). Clones PENP4 and PENP35 showed some degree of similarity to the Oscilliatorium, *Phormidium tenue*.

The majority (25%) of the BIS clones grouped with uncultured environmental bacteria (Table 2). Nevertheless, the uncultured clones were phylogenetically diverse and primarily mapped to the Actinobacteria, Acidobacteria, and α-Protebacteria. Three of the BIS clones, BIS 31 grouped with unclassified bacteria, rendering their taxonomical status undefined.

Discussion

Although PCR-based analyses of microbial diversity are widely acknowledged to be less than fully representative due to biases induced by factors such as extraction efficiency [17] and hybridization specificity [9, 45], it is also accepted that community composition is more effectively elucidated by this method than by conventional culturing.

Our results indicate that a diverse range of prokaryote phylotypes are present in Antarctic Dry Valley cold desert mineral soils. We note, as have others [28], that the highest proportion of sequences identified fall into the so-called “unculturable” class. A significant proportion of the sequences obtained showed relatively low homology to extant sequences (<95%), suggesting that the mineral soils of the Dry Valleys represent a substantial pool of novel species and/or genera. Calculations of diversity indices support previous suggestions that all three sample sites harbor relatively low species diversity (Table 3) [32, 48]. The calculated values, $H = 1.598$, 1.331, and 1.238 for PENP, MVG, and BIS, respectively, are substantially lower than would be expected for temperate soil biotopes, which typically have values of between 6 and 7 [16, 24]. However, we reiterate that this study only represents a snapshot of the microbial diversity present in three separate Dry Valley biotopes. It makes no attempt to fully assess microbial diversity or to imply which members of these microbial communities are functionally important.

Two of the dominant phyla, cyanobacteria and actinobacteria, identified in the oligotrophic mineral soil samples (MVG and PENP) are well represented in early culture-dependent studies of Antarctic Dry Valley mineral soil microbiology [6]. However, readily isolatable taxa, such as *Achromobacter*, *Bacillus*, *Corynebacterium*, *Micrococcus*, *Planococcus*, and *Pseudomonas* [6], are not

Table 3. Comparison of phylotype richness, diversity, and evenness values for the Antarctic mineral soil PENP, MVG and BIS bacterial communities

	16S rDNA clone libraries		
Index	*PENP*	*MVG*	*BIS*
S^a	49	50	57
H^b	1.598	1.331	1.238
E^c	0.410	0.340	0.306

[a]Phylotype richness, *S*, was calculated as the percentage of the total number of distinct ARDRA patterns to clones.
[b]Shannon–Weiner diversity index [16] was calculated as follows: $H = -\sum(\rho_i)(\log_2 \rho_i)$, where ρ is the proportion of an unique ARDRA pattern relative to the sum of all patterns.
[c]Evenness [16] was calculated form the Shannon–Weiner diversity function as follows: $E = H/H_{max}$ where $H_{max} = \log_2(S)$.

represented in any of the three 16S rDNA clone libraries. Conversely, groups such as Acidobacteria, Verricumicrobia, and Bacteroidetes, which are absent from both historical and recent culture-dependent studies, appear to be relatively common on the basis of 16S clone distribution. These groups have also been identified as significant components of the microbial communities in two previous molecular phylogenetic studies of specific Antarctic habitats: the cryptoendolithic [13] and cryoconite hole communities [10].

The appearance of cyanobacterial phylotypes as major contributors to only one of the three clone libraries supports the consensus that cyanobacterial distribution in the Dry Valleys is nonhomogeneous [50]. Water availability is generally assumed to be the principal factor dictating cyanobacterial distribution. For example, cyanobacteria are common in moist soils [34] and aquatic habitats including glacial streams [49] and flushes [22], saline lakes [39], and cryoconite holes [10]. Cyanobacteria also show a widespread but highly localized distribution in dry mineral soil habitats where suitable hypolithic strata are found [11; Cowan, unpublished results].

Sample PENP was recovered from desiccated surface gravels at 584 m altitude in a saddle between the Miers and Shangri La Valleys. There was no visible evidence of an adjacent cyanobacterial source (either algal mat material or hypolithic sites) at the time of sampling. The cyanobacterial phylotypes identified in sample PENP (putatively members of the genera *Nostoc*, *Phormidium*, and *Oscillatoria*) are also not indicative of cryptoendolithic origin, The cyanobacterial components of these communities are principally members of the *Gloeocapsa*, *Anabaena*, *Chroococcidiopsis*, *Hemichloris*, *Heterococcus*, and *Lyngbya* [36]. We also note that the hills on the northern flanks of the Miers Valleys appear to lack the sandstone and marble rock strata that principally harbor cryptoendolithic communities [36]. We thus suggest that the cyanobacterial signals identified in sample PENP constitute free-living cyanobacteria. Although the PENP sample showed a very low water content (0.7 wt.% H_2O) as determined by dry weight measurements, we suggest that such values may be a poor determinant of cyanobacterial distribution. Both meteorological studies [54] and surveys of lichen distribution [38] indicate that water availability is strongly altitude dependent. Relative humidity measurements from altitudinal transects in the Taylor and Wright Valleys [23] suggest that atmospheric humidity may be an important determinant of water availability. However, a detailed survey of cyanobacterial distribution in relation to environmental and physical factors (altitude, soil type, soil water content, soil humidity, and atmospheric humidity) is required to more fully understand the factors that dictate the distribution of soil-borne cyanobacterial populations.

The high frequency of actinobacterial phylotype signals (Table 2) suggests that this group of Gram-positive heterotrophic bacteria [4] contributes a significant fraction of the soil microbial population. However, few of the actinobacterial sequences could be matched to known phylotypes at >95% homology, suggesting that a substantial pool of novel uncultured psychrotrophic actinobacterial species remain to be identified. Given the industrial importance of this group of organisms [5, 26], this observation adds some weight to the importance of developing new isolation strategies [25].

Phylotypic signals identified as members of the acidobacteria were major components of both the desiccated mid-altitude (MVG) and C-enriched (BIS) samples. This group of microorganisms is widespread in soil biotopes showing widely diverse physical and chemical properties [3] and forms a major fraction of noncultured bacteria [44].

It has not been established whether the desiccated mineral soils of the Dry Valleys of Antarctica constitute stable microbial communities or merely assemblages of organisms attached to mobile particulates. The mobility of the mineral soils of the Dry Valleys (particularly during the windy austral winter seasons [54]) argues for the latter. A stable community is presumed to contain the elements of energy capture and turnover of primary nutrient components. Two of the three sites analyzed in this study may thus represent putative communities: the phototrophic and N_2-fixation capacity of the cyanobacterial phylotypes identified in sample PENP and the presence of an exogenous nutrient source in sample BIS offer the elements required to maintain a trophic structure. This cannot be said of the desiccated mineral soils of much of the Dry Valleys (represented by sample MVG in this study). Although certain phylotypic groups identified in sample MVG are known to include chemoautotrophic species (i.e., the β-proteobacteria [20, 42], the presence of specific chemoautotrophic species cannot be directly inferred from these results. The demonstration of a community structure may nevertheless be

inferred by identification of both the presence and activity of key genes, enzymes, and/or processes. These objectives form the basis for the future continuation of this study.

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