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Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica

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Abstract

A combination of culture-independent and culturing methods was used to assess the diversity of soil bacterial communities from four locations along 77 °S in Victoria Land, Antarctica. Soil samples were from the coast at Marble Point, in the Wright Valley from Bull Pass and near Lake Vanda, and from Mt. Fleming near the polar plateau. Total carbon and nitrogen, and water content of the soils were low, whereas total P was very high. The pH of the soils varied from extremely alkaline to slightly acid and electrical conductivity was medium to high on the coast and very high in inland soils from Bull Pass and Mt. Fleming. The average monthly air temperature was similar (−18 °C to −24 °C) at all the sites; however, in summer surface soil temperatures were >0 °C at Marble Point and in the Wright Valley for a total of 1100 and 1700 h, respectively. Marble Point soil had the most potential to support bacterial growth and activity with a mean total of 310 h per year when surface soils had a liquid volumetric soil moisture content >5%. Highest counts of culturable heterotrophs occurred in soil from Marble Point, whereas Mt. Fleming soil contained few organisms and had no liquid soil moisture recorded. Seven hundred and twenty-eight clones and 71 bacterial isolates were screened by restriction fragment length polymorphisms, and representatives of those dominant ribotypes that occurred more than 3 times were sequenced. The dominant ribotypes grouped within the bacterial divisions *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Thermus-Deinococcus*, *Acidobacteria*, *Firmicutes* and *Cyanobacteria*. The closest relatives of the amplicon library clones or cultured bacteria include the genera *Hymenobacter*, *Gillisia*, *Arthrobacter*, *Rubrobacter, Friedmanniella, Deinococcus* and *Leptolyngbya*. Many of the clones and bacteria were most similar to others from Antarctic sources, in particular a cyanobacterium-dominated cryptoendolithic community in Beacon sandstone. Some ribotypes were more prevalent in drier soils of the Wright Valley, including relatives of *Deinococcus*, *Rubrobacter* and clone FBP460 from Beacon sandstone. Bacterial communities from Marble Point soils were more diverse than those of the Wright Valley. Very few bacteria were isolated from Mt. Fleming soil.

Keywords: Bacterial diversity; Antarctic soil; Soil temperature; Soil water; Victoria Land

1. Introduction

In the virtual absence of plants, soils of Victoria Land, Antarctica, are microbially dominated ecosystems. The prevailing low temperatures, low humidity, freeze–thaw cycles, and salinity of soils combine to create a harsh environment for plant and animal life. Although few animals and plants have managed to colonise and survive in the soil, bacteria are distributed throughout.

Most investigations of bacteria in soils of Victoria Land have focused on the abundance and diversity of culturable bacteria in the Dry Valleys. Numbers of culturable bacteria in Dry Valley soils range from 0 to10^7 colony-forming units (CFU) g^{-1} dried soil (Boyd et al., 1966; Cameron et al., 1970). Numbers of bacteria in the subsurface soils from the

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moist layer above the ice-cemented layer were as high, or higher, than those in surface soils. Ecological factors that influence the distribution of culturable bacteria in the Dry Valleys include slope, drainage, exposure, available moisture, salt concentration and available nutrients (Cameron et al., 1970). Bacteria cultured from the soils were aerobic heterotrophs, with pigmented colonies prevalent on media inoculated with surface soils, and non-pigmented colonies dominating subsurface soils. In the absence of molecular tools, most of the bacteria were identified as Coryneforms belonging to the genera *Corynebacterium*, *Arthrobacter*, *Micrococcus* and *Brevibacterium*. *Bacillus* and the Gram-negative genera *Pseudomonas* and *Flavobacterium* were rare (Johnson et al., 1978). These studies led to the conclusion that bacteria from Antarctic soils maybe cosmopolitan, similar to those found in soils worldwide. The increased use of 16S rRNA gene sequencing and phylogenetic analysis is, however, beginning to provide new insights into the diversity of Antarctic bacteria and novel bacteria have been described from Dry Valley soils (Hirsch et al., 1998; Mevs et al., 2000).

A major limitation of bacterial diversity studies focused on culturable bacteria is that many bacteria present in environmental samples are not readily cultured using traditional approaches, hence diversity is underestimated. Culture-independent methods involving total community DNA extraction and characterisation of 16S rRNA gene amplicon libraries, now allow assessment of the composition of in situ bacterial communities without the need to culture. Application of DNA-based methods have greatly expanded knowledge of the bacterial composition of unique Antarctic ecosystems of the Victoria Land including lake ice (Gordon et al., 2000), microbial mats (Brambilla et al., 2001), cryptoendolithic communities (de la Torre et al., 2003) and cryoconite holes (Christner et al., 2003).

The objective of this study was to assess the bacterial community in soils of Victoria Land, in a range of soil-climate conditions, using a combination of culture-independent and traditional culturing methods. The focus of this study was the dominant bacteria in the soils. The soils were sampled from four locations along 77 °S latitude from the Antarctic coast inland along the Wright Valley towards the polar plateau. The bacterial diversity of the soils is related to local soil temperature and moisture regimes.

2. Materials and methods

2.1. Site location and analyses of soil

Soil samples were collected from four sites located near the coast at Marble Point, in the Wright Valley at Bull Pass, and near Lake Vanda (referred to throughout as Vanda) and on Mt. Fleming near the polar plateau (Fig. 1; Table 1). Pits were dug at the sites, and soil samples collected for analysis using an ethanol-swabbed trowel. The narrow distinct surface desert pavement layer, and the layer immediately below the surface were sampled except at the Vanda site where the narrow distinct surface layer plus 2 soil depths from deeper in the soil profile were sampled. The depths chosen were based on pedological descriptions of the soil materials (Table 1). Soil samples for chemical and microbial analysis were placed in sterile Whirl-Pak bags (Nasco), frozen at −20 °C, and transported to New Zealand for processing.

Soil samples were analysed for water content, pH, electrical conductivity, total phosphorus, and nitrate-N using standard methods (Blakemore et al., 1987). Total carbon and total nitrogen were determined using a Leco FP-2000 analyser.

For total microbial counts, cells in soil suspensions in 10 mM phosphate buffer (pH 7.2) were immobilised on

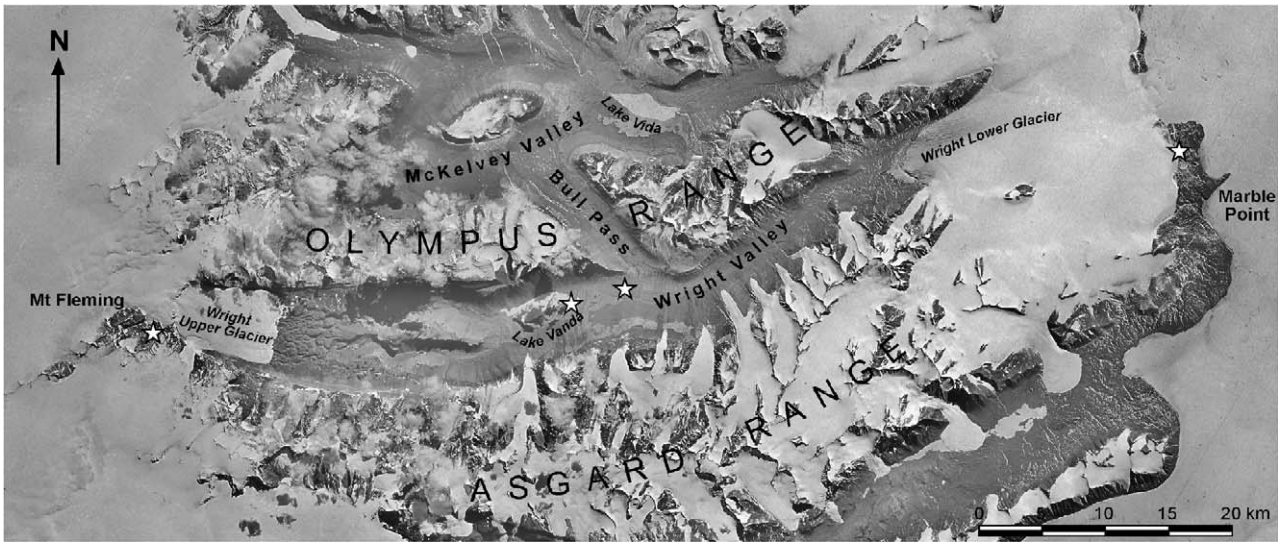

Fig. 1. Satellite photo showing the location of soil sampling sites (designated by a star) and surrounding areas (available from http://usarc.usgs.gov/antogc-viewer/declasdownload.htm).

Table 1
Geographical, geological and pedological characteristics of the different sites

Site Location	Marble Point	Near Lake Vanda	Bull Pass	Mt. Fleming
Coordinates	77°25′S 163°41′E	77°31′S 161°40′E	77°31′S 161°52′E	77°33′S 160°17′E
Site description	Marble Point former camp, 60 m E of lake edge. Slope of 3° to East, 50 m a.s.l.	On a bedrock ridge about 200 m southwest of the old Vanda Station and about 100 m SE of Lake Vanda, 296 m a.s.l.	400 m E of Bull Pass camp in Wright Valley. Slope of 4° to S, 152 m a.s.l.	Within Cirque at head of Wright Valley, on ablation moraine in very coarse patterned ground in the centre of a patterned ground polygon, 1697 m a.s.l.
Lithology	Marble dominated till with granite, dolerite, sandstone and gneiss	Granodiorite bedrock, weathered in situ with granite dominated till material	Granite dominated till with dolerite and sandstone present	Dolerite-dominated surface pavement over quartz and feldspar-dominated ablation till
Soil classification	Hypergelic Calcic Anhyorthel	Lithic Anhyorthel	Hypergilic Nitric Anhyorthel	Glacic Anhyorthel
Brief soil description	2 cm of desert pavement gravels over sandy gravel with >30% boulders/ stones. Ice-dominated horizon from 87 to 100+ cm	Soil material was formed in joints between large (20–80 cm diameter) granodiorite bedrock angular blocks. Soil material comprised gravely sand derived predominantly from granites, granodiorites and dolerite rocks	2 cm of gravel desert pavement over 1+ m of silt with a few large rocks. Visible salt accumulation on profile face. No ice cement	Strongly oxidised desert pavement of ventefacted boulders, through gravels, over 5 cm of bouldery gravely sand, over 40 cm of gravely silty fine sand, ice-cored moraine at 45 cm

gelatin-coated slides (Zarda et al., 1997), then stained with diphenylamidinoindophenol (DAPI) and counted using epifluorescence microscopy (Bottomley, 1994). Numbers of culturable heterotrophic microbes were determined by plating soil or soil dilutions (10^{-1}–10^{-4}) on R2A (Difco) agar plates with incubation at 15 °C for at least 6 weeks.

2.2. Soil climate

Soil climate stations with a data logger (CR10X Campbell Scientific Incorporated (CSI) Logan, Utah) were established at three of the sites (Marble Point, Bull Pass and Mt. Fleming). Soil temperature was monitored using thermistor temperature probes (Campbell 107, CSI) installed in triplicate at depths of 2 and 15 cm, in triplicate at Marble Point and Bull Pass, and with a single probe at Mt Fleming. Vitel soil water sensors (Hydrophobes, Stevens Water Monitoring Systems Inc.) were installed in triplicate at 2 and 20 cm depth at Marble Point, in duplicate at 2 and 20 cm depth at Bull Pass, and in triplicate at 7.5 cm depth at Mt. Fleming. The Vitel soil water sensors record both volumetric soil moisture content and temperature. Air temperatures were measured with a Campbell RM Young RTD air temperature probe with a Campbell HMP45L-L solar radiation shield attached, set 1.6 m above the ground. A Licor LI200X pyranometer solar radiation sensor was used to measure solar radiation 3 m above ground level. Soil humidity was measured at Bull Pass only using an "RH chip" supplied by NASA. Measurements were made every 20 min, averaged and recorded hourly, with data download annually. Data presented here were collected over the summers of 99/00, 00/01 and 01/02 for the Marble Point and Bull Pass sites and January 02 to January 03 for Mt. Fleming.

2.3. Bacterial community analysis

2.3.1. Bacterial DNA extraction and PCR amplification of 16S rRNA genes

The bacterial communities were examined using a combination of culturing and culture-independent methods.

Bacteria were selected from R2A plates used for enumerating culturable heterotrophs. The bacteria were selected to represent the different colony morphologies. Two to 6 replica plates provided 10–20 colonies per sample, except for Mt Fleming soil, which yielded only 2 bacterial colonies. Approximately 0.1 g of cell mass was recovered from the colony with a sterile pipette tip, and genomic DNA was extracted from bacterial colonies by a method previously described (Foght et al., 2004).

For the culture-independent methods, DNA was extracted from approximately 0.5 g of soil by mechanical cell disruption using zirconium beads as previously described (Foght et al., 2004). Bacterial 16S rRNA genes were amplified from extracted soil DNA (approximately 10 ng) by PCR using the oligonucleotide primers PB36 (5′-AGRGTTTGATCMTGGCTCAG-3′) and PB38 (5′-GKTACCTTGTTACGACTT-3′), corresponding to *Escherichia coli* positions 8–27 and 1492–1509, respectively (numbering per Brosius et al., 1981). PCR amplification was carried out using a program of 94 °C for 3 min

followed by 25–30 cycles of 94 °C, 45 s; 55 °C, 45 s; and 72 °C, 90 s. Duplicate PCR reactions were performed on each soil sample and the resulting products pooled and then purified using a High Pure PCR Purification Kit (Roche) according to the manufacturer's instructions. In addition, extraction with no soil, and reagent blank negative controls were carried out. Chimeric sequences were identified using CHIMERA_CHECK (version 2.7) of the Ribosomal Database Project.

2.3.2. 16Sr RNA gene amplicon library construction, restriction fragment length polymorphism analysis (RFLP) and sequencing of amplified 16S rRNA genes

Amplicon libraries were constructed in a T-tailed pGEM vector and introduced into *E. coli* DH5α as host strain. For each amplicon library, randomly selected colonies were prepared for RFLP analysis. The insets within the plasmid vectors were recovered by PCR from whole cells using vector-specific primers pGEM-F (5′-GGCGGTCGCGGGAATTCGATT-3′) and pGEM-R (5′-GCCGCGAATTCACTAGTGATT-3′). Amplification was carried out using the same program and conditions as described for primers PB36 and PB38.

The 16S rRNA genes of cultured isolates were amplified from cell lysates using extended primers PB36E (5′-TTTTTTTTTTTTTTTGGAGRGTTTGATCMTGGCTCAG-3′) and PB38E (5′-CCKCAKCTTCCGGTKGGGKTACCTTGTTACGACTT-3′). The 3′ ends of these primers are the same as PB36 and PB38. The purpose of the long 5′ extensions is to allow direct RFLP comparison between these amplicons and those generated from the clones where the insets were amplified using vector-specific primers.

The PCR products from clone libraries and from amplified cultured isolates were screened for similarity by RFLP analysis using *Hae*III digestion. Fingerprint profiles were compared for similarity using the program GelCompar II (Version 2.2 Applied Maths; Kortrijk, Belgium). Any ribotype containing three or more members was selected for DNA sequencing; a ''ribotype'' being defined as any unique pattern of DNA fragments found when the amplified 16S rRNA genes of either library clones or isolated cultures were digested with *Hae*III. One to 5 representatives of each ribotype were sequenced except for ribotypes E and AB, which had the same pattern as sequences *Hymenobacter* 34/38 (GenBank accession No. AY571818) and *Sporosarcina* 34/47 (GenBank accession No. AY571844) respectively (Saul et al., 2005). Partial 16S rRNA gene sequences were compared to known sequences in GenBank (http://www.ncbi.nlm.nih.gov) using the BLAST search tool.

2.3.3. Phylogenetic analysis of Bacteroidetes

Because of their prevalence in Victoria Land soil the *Bacteroidetes* were chosen for phylogenetic analysis. In addition to sequences for ribotypes A–K, sequences were selected from GenBank on the basis of the BLAST results and supplementary taxa were added. Taxa were chosen on the basis of relevance to the study and sequence quality. Named organisms were included in the tree to act as phylogenetic markers, and other taxa were included that helped to break long branches. All phylogenetic analyses were performed using PAUP* (Swofford, 2002). Because of the number of taxa, the Neighbour Joining algorithm was used to construct the phylogenetic trees. Pairwise distances were estimated using the GTR + Γ + Proportional of Invariant sites (PI) method of evolution with parameters estimated from the data. Bootstrap values were calculated using the same method with 2000 replicates using the same model of evolution.

2.3.4. Characterisation of cultured bacteria

Eight bacterial cultures representative of ribotypes C, D, G, L, M, P, U and AA were characterised. Gram stains, oxidase and catalase tests were determined on cultures grown on R2A using standard methods (Gerhardt, 1994). Metabolism of carbon substrates was determined on API 20NE strips (Biomerieux, France) inoculated as per manufacturers' instructions and incubated at 15 °C for 7–14 days. In addition, growth on 5-mM trehalose, glycerol and *d*-arabitol was determined on Bushnell Haas minimal medium (Difco) solidified with 1.5% purified agar (Difco). The plates were inoculated with $4 \times 10\,\mu L$ drops of cell suspensions in 10mM phosphate buffer (pH 7) prepared using young cultures and incubated at 15°C for 2 weeks. Growth was compared with that on minimal media prepared without additional carbon source. APIZYM test strips (Biomerieux, France) were used to screen isolates for hydrolytic enzymatic activities and inoculated as per manufacturers' instructions. After inoculation, the test strips were incubated at 15 °C for 16 h before results were recorded. In addition, hydrolysis of DNA was determined on DNAse Agar (Difco).

Growth at 5, 15 and 25 °C was determined on R2A plates. Salt tolerance was determined using R2A plates amended with NaCl (0%, 1%, 2%, 5% and 10% w/v). All plates were inoculated with young cell suspensions as described above, and incubated at 15 °C for 2 weeks.

3. Results

3.1. Soil characteristics

The soils all had gravely sand textures, except the Bull Pass subsoil which was silt dominated, and the moisture content of all soil samples was low (Table 2). The pH of the soils varied from extremely alkaline in soil from Marble Point and Vanda, to neutral to slightly alkaline at Bull Pass, and neutral to slightly acidic at Mt. Fleming. Total carbon and nitrogen were very low, whereas total P was very high. High nitrate levels in soil from Mt. Fleming qwere attributed to the accumulation of salt. Electrical

Table 2
Selected chemical, physical, and microbiological properties of soil samples analysed for bacterial diversity studies

Sample location	Depth (cm)	Moisture (%)	pH	EC (mS/cm)	Total C (%)	Total N (%)	NO_3-N (mg/kg)	Total P (%)	Total direct counts (g^{-1} dry soil)	Number of culturable heterotrophs (g^{-1} dry soil)
Marble Point	0–3	2.4	9.6	0.65	0.28	0.02	2.2	0.069	1.3×10^8	3.7×10^5
Marble Point	3–15	5.3	9.0	1.23	0.11	0.01	5.9	0.068	7.8×10^7	8.6×10^4
Bull Pass	0–2	0.2	7.6	5.58	0.03	0.01	0	0.022	2.3×10^7	5.6×10^3
Bull Pass	2–5	1.0	7.1	7.94	0.02	0.01	2.9	0.023	$<10^6$	8.4×10^3
Vanda	0–2	0.2	9.1	0.09	0.06	0.00	1.3	0.020	1.2×10^8	3.6×10^4
Vanda	5–10	0.3	8.5	0.31	0.05	0.01	6.4	0.020	6.6×10^7	2.1×10^4
Vanda	25–30	0.5	8.0	0.43	0.03	0.00	13.2	0.020	3.6×10^7	1.7×10^3
Mt. Fleming	0–1	1.8	7.1	3.56	0.03	0.08	721	0.016	$<10^6$	<10
Mt. Fleming	1–5	4	6.2	4.35	0.01	0.08	613	0.015	$<10^6$	<10

conductivity ranged from low or very low at Vanda to very high in soils from Bull Pass and Mt. Fleming.

Total microbial counts were highest in surface soils from Marble Point and Vanda ($1.2–1.3 \times 10^8\,g^{-1}$ dry soil) and below detection limits ($<10^6\,g^{-1}$ dry soil) in soil from Mt. Fleming and Bull Pass subsurface. Highest numbers of culturable heterotrophic microbes were detected in surface soil from Marble Point ($3.7 \times 10^5\,CFU\,g^{-1}$ dry soil), with fewer detected in Wright Valley soils (5.6×10^3 and $3.6 \times 10^4\,CFU\,g^{-1}$ dry soil) from Bull Pass and Vanda, respectively, and even fewer in Mt. Fleming soils ($<10\,CFU\,g^{-1}$ dry soil). A smaller proportion of the microbes counted in Vanda surface soils were culturable on R2A agar compared with those in Marble Point surface soil.

3.2. Soil temperature and moisture regimes at sample sites

While solar radiation was similar at all three sites, both air and soil temperatures were warmer at the Marble Point and Bull Pass sites than at the higher altitude site at Mt. Fleming (Table 3). Mean annual soil temperatures at the sites were low ($<-17\,^{\circ}C$; Table 3). However, during summer months (December–February) when there is continuous daylight, average soil temperatures were $>0\,^{\circ}C$ at Marble Point and Bull Pass (Table 3; Fig. 2). Soil temperatures in summer showed diurnal variations (Fig. 2) that are strongly correlated with incoming solar radiation (Balks et al., 2002).

Marble Point had the longest period with volumetric liquid soil moisture content greater than 5%, and no liquid moisture was recorded at Mt. Fleming as the soil temperature did not rise above freezing at the depth of the moisture measurement (7.5 cm) (Table 3).

The longest period of soil temperatures greater than $0\,^{\circ}C$ and the most freeze/thaw events were recorded at the Bull Pass site. However, in the 3 years monitored at Bull Pass there was only one soil moistening event (in December 1999) when near-surface volumetric soil moisture at one of the two replicate sensors rose rapidly to 5% then declined to the background level of about 1% over a period of about 1 week. The other replicate showed no change. The wetting event was likely to have been due to local melting of a small snowdrift and did not extend to the 20 cm depth where volumetric soil moisture contents remained consistently at about 4% throught-out the summer.

3.3. Analysis of bacterial clone libraries and bacterial isolates, and compilation of sequence data

Seven hundred and twenty-eight amplicon library clones and 69 bacterial isolates from Marble Point, Bull Pass, and Vanda and 2 isolates from Mt. Fleming (no clones obtained) were screened by restriction fragment length polymorphisms (RFLP). All clones and bacteria with unique restriction patterns are referred to as ribotypes. Between 44% and 85% of the clones were assigned to dominant ribotypes that occurred 3 or more times. Table 5 summarises the distribution of the dominant ribotypes sorted by phylogenetic division. All the ribotypes sequenced had greater than 88% similarity to 16S rRNA gene sequences available in GenBank (August 2005), 16 were more than 95% identical. The Shannon–Weaver indices calculated for the clone libraries (Shannon and Weaver, 1949) indicate that bacterial communities in surface soil from Marble Point (3.95) are more diverse than those of Bull Pass or Vanda in the Wright Valley (<3.27). The diversity of the Victoria soil bacterial communities was comparable to that of Scott Base (3.76 and 3.70) but less than that reported for arid Arizona soils (6.61–7.09) (Dunbar et al., 1999). It should be noted, however, that the low coverage (Chao and Lee 1992) of the Marble Point and Scott Base soil clone libraries (0.59–0.49; Table 4 and Saul et al., 2005) compared with those from the Wright Valley (>0.69; Table 4) can lead to underestimates of the diversity of the soil bacterial communities (Hill et al., 2003).

3.4. Diversity of dominant soil bacteria

The clones or isolates sequenced grouped with the recognised bacterial divisions *Bacteroidetes*, *Actinobacteria*,

Table 3
Mean climate data for Marble Point, Bull Pass, and Mt. Fleming

	Marble Point[a]	Bull Pass[a]	Mount Fleming[b]
Temperature (°C)			
Mean annual			
Air	−18	−20	−24
Soil, 2 cm depth	−18	−20	−23
Soil, 15 cm depth	−18	−20	−23
Mean January			
Air,	−3	−0.4	−12
Soil, 2 cm depth	+3	+5	−7
Soil, 15 cm depth	+2.5	+3	−8
Mean July			
Air	−24	−29	−32
Soil, 2 cm depth	−27	−33	−35
Soil, 15 cm depth	−27	−32	−33
Soil relative humidity (%)			
Mean annual	ND[c]	33	ND
Mean January	ND	39	ND
Mean July	ND	36	ND
Solar radiation ($W m^{-2}$)			
Mean annual	120	101	118
Mean January	300	270	295
Mean July	0	0	0

	2 cm	20 cm	2 cm	20 cm	2 cm	7.5 cm
Hours with soil temperature >0 °C	1100	850	1700	1300	13	0
Freeze/thaw events per summer (number)[d]	52	24	79	27	1	0
Total hours of liquid water >5% per summer (h)[d]	310	48	1	0	ND	0
Maximum volumetric liquid soil moisture content (%)[d]	32	7	5.4	4.6	ND	0
Soil moistening events per summer (number)[d]	2	1	0.2	0	ND	0

[a]Marble Point and Bull Pass data are the means of data from 1999 to 2003 (except those below)
[b]All Mt. Fleming data are for the 2002 year only
[c]ND = not determined
[d]Data from Marble Point and Bull Pass, at 2 cm and 20 cm depth, were averaged over three summers (1 Nov–28 Feb 99/00, 00/01, 01/02) (after Wall 2004).

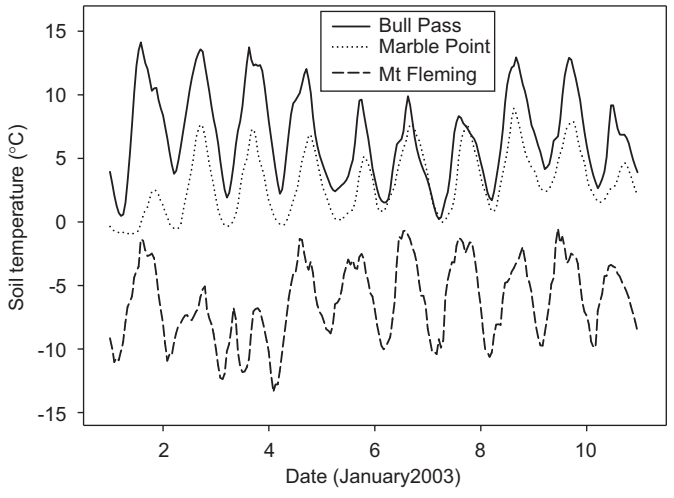

Fig. 2. Example of soil temperature at 2 cm depth measured continuously for 10 days in January 2003.

Proteobacteria, *Thermus-Deinococcus, Acidobacteria*, *Firmicutes* and *Cyanobacteria* (Table 5 and Fig. 3). Eleven of the ribotypes grouped within the *Bacteroidetes* and 7 within the *Actinobacteria*. Three or less ribotypes were assigned to remaining divisions with single ribotypes assigned to each of the alpha, beta and gamma *Proteobacteria*. Twenty-four, 22 and 20 of the ribotypes were present in soil from Bull Pass, Marble Point and Vanda, respectively.

A feature of the bacterial communities in soils from Marble Point, Bull Pass and Vanda was the dominance of a few ribotypes with relative abundances >0.1 (Fig. 3). *Proteobacterium* T was dominant at all sites, *Proteobacterium* U was dominant in Bull Pass and Marble Point soils, and *Bacteroidetes* I, *Actinobacteria* O and *Bacteroidetes* B were each dominant at Vanda, Bull Pass and Marble Point, respectively.

The *Bacteroidetes* ribotypes A and C were only detected in Vanda soil whereas F*irmicutes* ribotype AA was unique

Table 4
Number of clones, ribotype patterns, percent clones assigned to abundant ribotypes that occurred more than 3 times, Shannon–Weaver Indices and estimates of sampling coverage for each sample site

Sample location		No. of clones	No. of ribotype patterns	Clones assigned to abundant ribotypes (%)	Shannon- Weaver indices	Sampling coverage (%)
Marble Point	Surface	145	85	56	3.95±.005	0.50
	Subsurface	94	52	44	2.65±0.018	0.52
Bull Pass	Surface	144	47	82	3.19±0.006	0.81
	Subsurface	92	29	85	2.53±0.01	0.83
Vanda	Surface	113	47	69	3.27±0.009	0.70
	subsurface[a]	140	61	67	3.32±0.008	0.70

[a]For simplicity clone libraries for subsurface soils from Vanda were combined.

to Marble Point soil. The distribution of some ribotypes varied within the soil profile. Notably, Ribotypes U and I were present in the surface and subsurface of the Wright Valley soils and in the surface only at Marble Point. Similarly, T was prevalent in Marble Point subsurface soil and Bull Pass surface soil. *Actinobacteria*, in particular ribotypes O and P, and *Thermus-Deinococcus* (V, W and X) were more prevalent in clone libraries of the Wright Valley soils, whereas the *Proteobacteria* were prevalent in all clone libraries (Fig. 3).

The closest relatives of some of the ribotypes were from various Antarctic sources including cryptoendolithic communities (A, C, N, P, V, W, X), soil (E, G, O), microbial mat (Z), lake water (R) and sea ice brine (M) (Table 5).

Table 6 lists the number of those bacterial isolates assigned to each division. Most of the isolates assigned grouped within *Arthrobacter* (L and M) and the gamma *Proteobacteria* (U) with single isolates assigned to ribotypes C, G, AA and P.

3.5. Phylogenetic analysis of bacteroidetes

The *Bacteroidetes* dominant in the soils grouped with the genera *Gillisia* (B) belonging to the family Flavobacteriales, and *Hymenobacter* (E, F and G), *Pontibacter* (A, C and D) and *Flexibacter* (H, I, J and K) within the family Flexibacteriales (Fig. 4). Ribotype B clustered with a number of marine isolates, including Abyssal str. AIII4 and *Gillisia mitskevichiae* KMM 6034^T, and was prevalent in coastal Marble Point soil but not that of the Wright Valley. Ribotypes E (identical to *Hymenobacter* 34/38 from Scott Base) and G clustered with *Hymenobacter roseosalivarius* AA688 from soil from Linneaus Terrace in Victoria Land, and *Hymenobacter* 35/26 from Scott Base soil. Ribotypes A, C and D clustered with bacteria identified as *Pontibacter actiniarum* and *Sedimentibacter rubrus*. Ribotype C was very closely related to cytophagales clone FBP292 from a cyanobacterium-dominated cryptoendolithic community in Beacon Sandstone, Victoria Land. There are no close cultured relatives of ribotypes H, I, J and K. They are all only distantly related to uncultured *Bacteroidetes* clone D132.

3.6. Characterisation of isolates

Bacteria belonging to ribotypes C, D, G, L, M, P, U and AA were isolated during this study and sole representatives of each were characterised (Table 7). The isolates are referred to as Isolate C–AB, respectively. The isolates included both Gram-positive and -negative organisms and were rods or coccibacilli. Many of the isolates formed pigmented colonies on R2A agar plates. The isolates varied with respect to the carbon sources used for growth. The *Arthrobacter* isolates (ribotypes L and M) used the widest range of carbon sources, whereas remaining strains used less than three, and no isolates grew on adipate, caprate or citrate.

All isolates were catalase positive but varied with respect to oxidase. All isolates were positive for phosphatase activity and only Isolate P exhibited lipase activity. The *Bacteroidetes* isolates (C, D and G) and the *Proteobacteria* isolate (U) were most active and exhibited phosphatase, proteolytic and glycosyl-hydrolase activity. Only Isolate AA produced DNAse on DNAse agar plates.

The isolates are psychrotolerant. They were isolated at 15 °C, some grew at 25 °C (C, G, M, U and AA), and some at 5 °C (Isolates C, L, M, U and AA). Similarly, their response to salinity varied. The most salt tolerant isolates were Isolates M, P and AA, whereas U and G did not grow with 1% NaCl.

4. Discussion

Using both culture-independent and culturing techniques we assessed the diversity of bacterial communities in soils from four locations of Victoria Land, Antarctica (Fig. 1) with a view to elucidating the dominant bacteria. While all techniques designed to assess diversity are inherently biased, the combination of culture-based and DNA-based approaches add substantially to our knowledge of the bacterial diversity of Victoria Land soil. Clone

Table 5
Taxonomic affiliation of ribotypes defined by RFLP analysis of amplified 16SrRNA gene sequences digested with *HaeIII* and DNA sequencing of the genes

Ribo-type	Clone/Isolate sequenced	GenBank Accn no.	Closest BLAST match (GenBank accession no.) (source) (% identity)
Bacteroidetes			
A	J35H2	DQ365987	Uncultured cytophagales bacterium clone FBP292 (AY250875) (Cryptoendolithic community, Antarctica) (92%)
B	2C10	DQ365988	*Gillisia mitskevichiae* (AY576655) (Seawater, Sea of Japan) (98%)
C	J35G6	DQ365989	Uncultured cytophagales clone FBP292 (AY250875) (cryptoendolithic community, Antarctica) (99%)
D	20/6	DQ365990	*Pontibacter actiniarum* (AY989908) (unpublished) (95%)
E	34/38[a]	AY571818	*Hymenobacter roseosalivarius* strain AA688 (Y18834) (Soil, Antarctica) (99%)
F	MP1-9C	DQ365991	Uncultured Bacteroidetes CSR-16 (AY699368) Intertidal hot-springs Iceland (89%)
G	21/4	DQ365992	*Hymenobacter roseosalivarius* strain AA688 (Y18834) (Soil, Antarctica) (98%)
H	J35E6	DQ365993	Uncultured Bacteroidetes clone TH451 (AJ888562) (Taihu Lake, China) (92%)
I	21A5, 21A6, J34D9, J34E2 & J35A3	DQ365994	Uncultured Bacteroidetes clone S1-4-CL9 (AY728066) (decayed velvet leaf seed) (91%)
J	BP20 2e	DQ365999	Uncultured Bacteroidetes clone D132 (AY274138) (Contaminated mine tailings) (94%)
K	21A8	DQ366000	Uncultured bacterium clone 47 mm29 (AY796035) (gold mine bore water, South Africa) (90%)
Actinobacteria			
L	20/2	DQ366001	*Arthrobacter* sp. Fa21 (AY131225) (phyllosphere of a strawberry plant) (98%)
M	20/4	DQ366002	*Arthrobacter* sp. S23H2 (AF041789) (Sea ice brine, Antarctica) (99%)
N	21D4	DQ366003	Uncultured actinobacterium clone FBP402 (AY250880) (Cryptoendolithic community, Antarctica) (95%)
O	21F8	DQ366004	Uncultured *Rubrobacter* sp. clone 354H (AY571811) (Soil, Antarctica) (95%)
P	20G6	DQ366005	Uncultured actinobacterium clone FBP460 (AY250884) (Cryptoendolithic community, Antarctica) (96%)
Q	21F7 & J34G3	DQ366006	*Friedmanniella* sp. Ellin171 (AF409013) (Soil, Australia) (97%)
R	20H4	DQ366008	*Friedmanniella* sp. EL-17a (AJ132943) (Ekho Lake, Antarctica) (98%)
Proteobacteria			
S	20C11	DQ366009	Alpha-proteobacterium Bacterium Ellin6055 (AY234707) (Soil, Australia) (96%)
T	20F1	DQ366010	Uncultured beta-bacterium clone RA7C9 (AF407379) (Contaminated groundwater, Germany) (99%)
U	MP1-2H	DQ366011	Uncultured gamma-proteobacterium BD3-1 (AB015547) (Deep sea sediments, Ryukyu Trench) (92%)
Thermus-Deinococcus			
V	21D1 & 20E7	DQ366012	Uncultured *Deinococcus* bacterium clone FBP266 (AY250871) (Cryptoendolithic community, Antarctica) (97%)
W	21B7	DQ366014	Uncultured *Deinococcus* bacterium FBP266 (AY250871) (Cryptoendolithic community, Antarctica) (97%)
X	J34H5 & 21B1	DQ366015	Uncultured *Deinococcus* bacterium FBP266 (AY250871) (Cryptoendolithic community, Antarctica) (98%)
		DQ366016	
Acidobacteria			
Y	20G5	DQ366017	Uncultured soil bacterium clone DS-13 (AY289372) (Surface soil, USA) (90%)
Cyanobacteria			
Z	20B9	DQ366018	Uncultured cyanobacterium (AY151727) (Microbial mat, Lake Fryxell, Antarctica) (97%)
Firmcutes			
AA	34/47[a]	AY571845	Uncultured bacterium clone AKAU3733 (DQ125665) (uranium-contaminated soil, USA (99%)

All ribotypes in bold are those for which we have obtained cultured representatives in this investigation.
[a]From Saul et al., (2005).

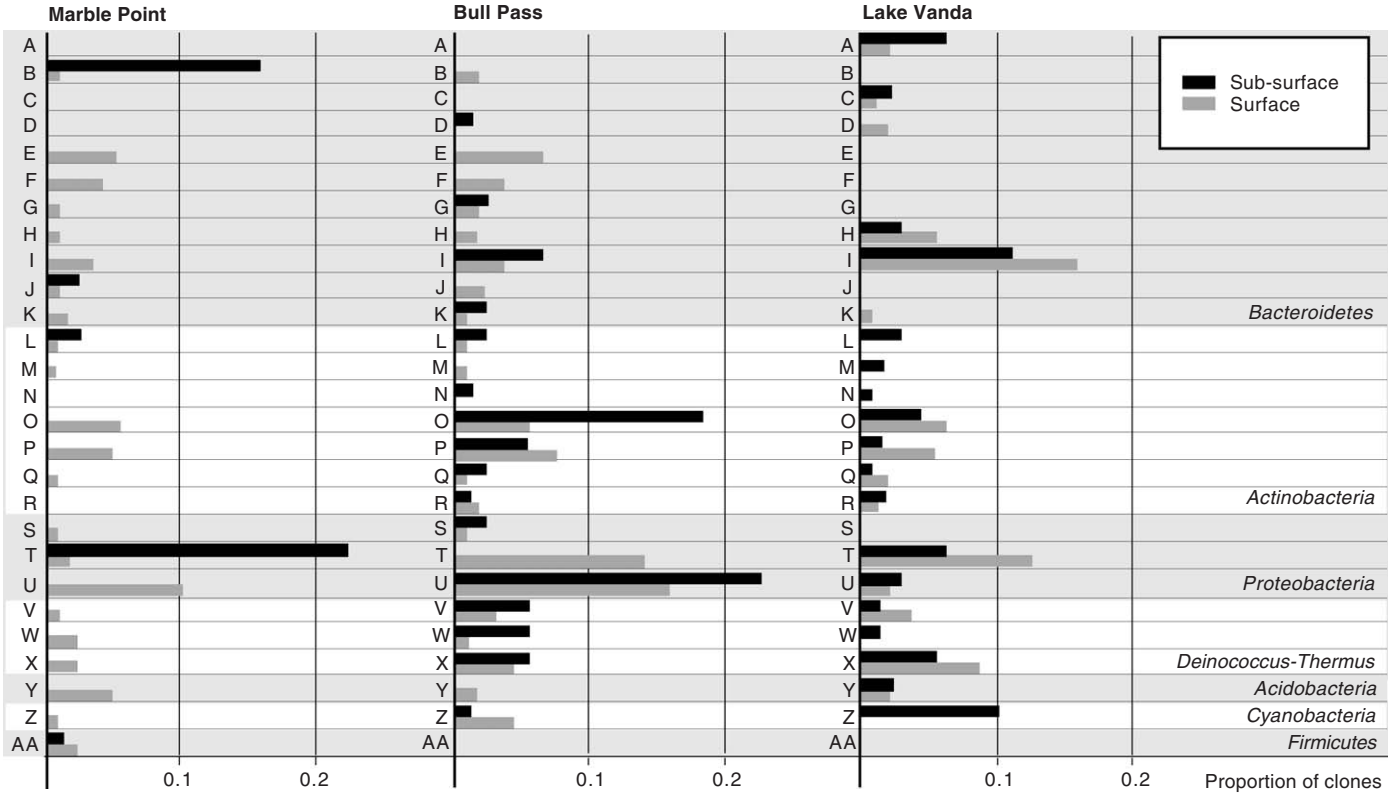

Fig. 3. Histograms depicting the relative abundance of the major divisions of Bacteria detected in surface and subsurface soil from Marble Point, Bull Pass and Vanda by culture-independent methods. Clones found in low abundance are excluded from the histogram.

Table 6
Source and number of bacterial isolates grouped within ribotypes

Ribotype	Source[a]		
	Marble Point	Bull Pass	Vanda
C			1
D		2	1
G		1	
L	3	3	7
M	6	1	4
U		5	4
AA		1	

[a]A single isolate from Mt. Fleming soil grouped with ribotype P.

libraries, for example, are biased by differential extraction of DNA and PCR amplification, and by the longevity of naked DNA in Antarctic soils (Tow and Cowan, 2005). Similarly, isolation media and incubation conditions are highly selective. A DNA-based approach can provide a semi-quantitative estimate of the relative abundance of different groups of bacteria within a community, whereas culturing reveals the organisms that are viable and provides appropriate organisms for investigations of bacterial adaptation to cold, desiccation and freeze–thaw. Furthermore, bacterial diversity assessment in combination with site chemical and climate data allows speculation on the influence of soil conditions on the soil bacterial community structure.

A feature of the structure of the bacterial communities of soil from Marble Point, Bull Pass and Vanda in Victoria Land was the dominance of a few ribotypes that occurred more than three times in the respective clone libraries and varied between sites. Furthermore, two or three of the ribotypes from each location had a relative abundance >0.1 (Fig. 3). Such dominance is highly unusual for surface soils (Zhou et al., 2002), but may be a feature of Antarctic terrestrial ecosystems (de La Torre et al., 2003; Saul et al., 2005). Dominant bacterial ribotypes or sequences were detected in soil from Ross Island (Saul et al., 2005) and in a cryptoendolithic community in Beacon Sandstone (De la Torre et al., 2003). In contrast, duplicate sequences were rare in clone libraries recovered from Wisconsin agricultural soil (Borneman et al., 1996), Amazon forest and pasture soil (Borneman and Triplett, 1997), Siberian Tundra soil (Zhou et al., 1997), sandy soils from Virginia, Delaware and Michigan (Zhou et al., 2002), and Colorado alpine soil (Lipson and Schmidt, 2004). Zhou et al. (2002) proposed that dominant bacteria in soils are more competitive at utilising available resources and are better adapted to in situ conditions. Victoria Land soils are cold desert soils characterised by low temperatures and low moistures. Additional stresses for microbial growth and activity include low nutrients and alkaline pH

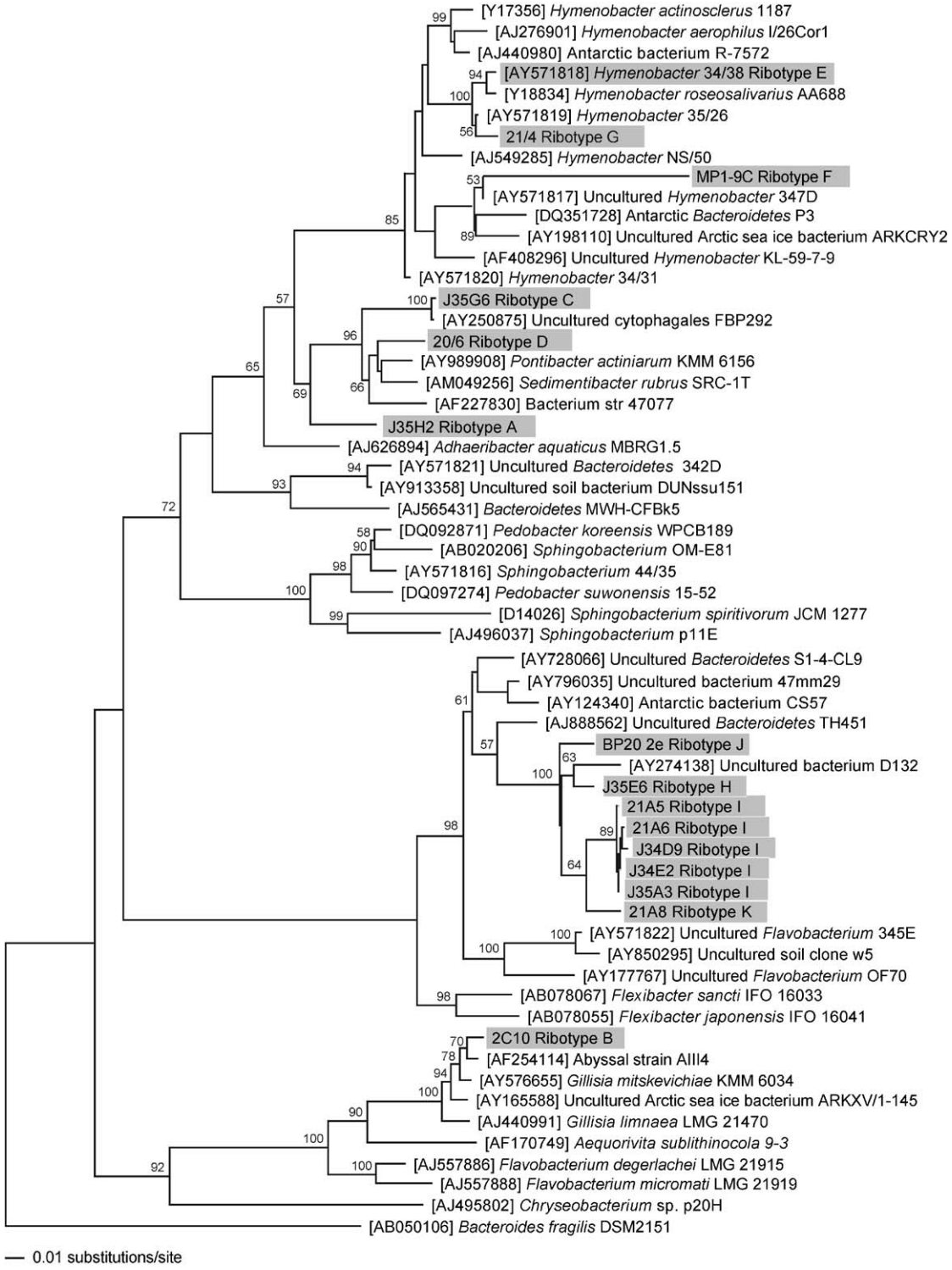

Fig. 4. Neighbour Joining tree of the *Bacteroidetes* from Victoria Land soil. The tree was constructed from 1401 sites of the aligned 16S rRNA genes and rooted with the sequence from *Bacteroides fragilis*. Numbers above the branches are bootstrap values estimated from 2000 re-sampling replicates. Where a clade has no associated number the bootstrap values are below 50%.

(especially for coastal soils like those of Marble Point). However, the main influences on heterotrophic bacterial growth and activity in these soils is likely to be the availability of organic carbon and liquid water (Barrett et al., submitted).

Compared with temperate soils, carbon and nitrogen resources in Victoria Land soils are typically low and often patchy (Table 1; Hopkins et al., 2005). Hence bacteria in these soils would be advantaged if they had high-affinity nutrient uptake systems that allowed growth at

Table 7
Characteristics of bacteria assigned to dominant ribotypes

Characteristics	Ribotype C	D	G	L	M	P	U	AA
Division Genera (if known)	*Bacteroidetes*	*Bacteroidetes*	*Bacteroidetes* *Hymenobacter*	*Actinobacteria* *Arthrobacter*	*Actinobacteria* *Arthrobacter*	*Actinobacteria*	*Proteobacteria*	*Firmicutes*
Strain ID	36/10	20/6	21/4	20/3	M2(3)	Fl(4)	20/5	D1
Colony pigmentation	Pink	Pink	Pale pink	Bright pink	Cream	Cream	Cream-pink	Cream with yellow/brown diffusible pigment
Cell shape	Short plumb rods, coccibacilli	Short plumb rods, some longer	Short plumb rods, some longer	Cocci in sheets	Small pointy rods	Coccibacilli	Rods, some longer, slightly bent	Large straight rods, some in pairs
Gram reaction	–[a]	–	–	+[a]	+	+	–	+
Growth substrates								
NAG[b]	–	–	–	+	+	–	–	–
Arabinose	–	–	–	+	–	–	–	–
d-arabitol	–	–	–	–	+	–	–	–
Gluconate	–	–	–	+	+	+	–	–
Glucose	–	–	–	+	+	+	–	–
Glycerol	–	–	–	–	+	–	–	–
Malate	–	–	–	+	+	–	–	–
Mannitol	–	–	–	+	+	–	–	–
Maltose	+	–	–	+	+	–	–	–
Mannose	–	–	–	+	+	–	–	–
Phenylacetate	–	–	–	–	+	–	–	–
Trehalose	–	–	–	–	+	–	+	–
Enzymatic activities								
Oxidase	+	+	+	–	–	–	+	–
API ZYM Test[c]								
Phosphatase	++	+++	+++	+	++	++	+++	+
Lipase	–	–	–	+	–	+	–	–
Protease	++	++	++	+	+	–	+	–
Glycosidase	+	++	+	+	+	–	++	–
Temperature[d] range (°C)								
5, 15 & 25	+++	–+–	–++	++–	+++	– + –	+++	+++
Salinity [e](w/v %)								
1, 2, 5 & 10	+–––	+ +––	––––	+ +––	+++ –	+++ –	––––	+++ –

[a]Where NAG means *N*-acetylglucosamine.
[b]Where "+" means growth and "–" means no growth enhanced.
[c]Enzymatic activities as revealed by APIZYM tests, scored on an arbitrary scale from "+" increasing to "+++".
[d]Where "+++" means growth at 5, 15 and 25 °C, "–+–" means growth at 15 °C only, "++–" means growth at 5, and 15 °C and –++ means growth at 15 and 25 °C.
[e]Where "––––" means no growth, "+–––" means growth at 1% salt, "++––" means growth and 1% and 2% salt, and "+++–" means growth at 1%, 2% and 5% salt.

low-substrate concentrations when conditions are favourable (Vincent, 2000). Sources of organic carbon include (i) modern in situ autotrophic activity by cryptoendolithic communities or soil microbes, (ii) legacy organic deposits from ancient lake beds and (iii) spatial subsidies from coastal regions or microbial mats from modern lakes carried to the soils by aeolian dispersal (reviewed in Hopkins et al., 2005). The higher carbon content of the coastal Marble Point soil may be due to extensive cyanobacterial mats that form on the margins of a stream near (<100 m) the soil sample site. In laboratory experiments a large proportion of the Dry Valley soil C and N was shown to be mineralisable (Barrett et al., 2005). Among the readily degradable carbon compounds extracted from cryptoendolithic communities in sandstone were glucose, arabitol, succinate, oxalate and the amino acids, alanine and arginine (Siebert et al., 1996). These compounds could be released to soil following wind erosion. In addition to windblown material from microbial mats or cryptoendolithic communities (Hopkins et al., 2005), nitrogen is deposited in soils as nitrate from atmospheric deposition (Wada et al., 1981). High concentrations of nitrate in Mt. Fleming soil (Table 1) may be due to consistently freezing conditions (Table 3) leading to negligible leaching rates and low rates of in situ microbial activity. The Mt. Fleming soil was developed on a much older surface than the other sites, as evidenced by much greater weathering of desert pavement rocks and the higher elevation, so would have had a much longer time for nitrate to accumulate in the soil.

For bacteria to survive and be active in Victoria Land soils they will need to be cold tolerant and resistant to freeze-thaw, which can be lethal. In addition, bacteria in the soils may be exposed to desiccation due to low water availability and low soil humidity.

At low water contents, osmotic and matric forces limit the availability of water and consequently bacterial growth and activity. At a volumetric moisture content of 5%, the matric potential of both Marble Point and Bull Pass soil materials is generally greater than −1500 kPa (Balks et al., 2002). Furthermore, the soil EC is medium in surface soil, hence the soil salts are not likely to impact negatively on water availability. Thus it is likely that water is available for microbial activity at volumetric soil moisture contents $\geqslant$5%. However, it is possible that some organisms, particularly hyperxerophyllic microbes, may operate at water potentials as low as −8000 kPa (Skujins, 1984).

The soil climate data (Table 3) suggest that, with the longest period of liquid volumetric moisture content greater than 5%, conditions at Marble Point would be more conducive to microbial survival and activity than conditions at the other sites. In the Wright Valley, although precipitation falls as snow much of it is lost to sublimation without the opportunity for moisture to penetrate into the soil. The fine (silt-dominated) textures of the Bull Pass subsoils will have a lower moisture availability than the other gravelly sand-dominated soils for the same moisture content. Conditions at Vanda may be moister than those at Bull Pass due to proximity to Lake Vanda. Furthermore, the lower EC of the Vanda soils, compared with that of Bull Pass soil, indicates salts may have been leached from the Vanda soils or the soils possibly contain ''less weathered'' parent materials. In contrast to Marble Point and the Wright Valley sites, conditions on Mt. Fleming are more severe for bacterial activity. Minimal liquid water is available at this site due to freezing temperatures, and what little was available would have had high osmotic potential as a result of high salts concentration in the soil (Table 2).

The dominant clones and bacteria retrieved from the Victoria Land soils belong to the bacterial divisions *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Deinococcus-Thermus*, *Acidobacteria*, *Cyanobacteria* and *Firmicutes*. Except for *Deinococcus-Thermus* and *Cyanobacteria*, they are among the divisions commonly reported in soil (Buckley and Schmidt, 2001; Zhou et al., 2003). Other groups common in soil are *Verrucomicrobia* and *Planctomycetes*. While they were not dominant in the soils we analysed they have been detected previously in Antarctica. *Verrucomicrobia* have been detected in microbial mat material from Lake Fryxell (Brambilla et al., 2001) and *Planctomycetes* in soil from the Vestfold Hills (Smith et al., 2000).

Of the 27 ribotypes that are dominant in Victoria soils 11 belonged to the *Bacteroidetes* (Table 5). *Bacteroidetes* are have been reported in Antarctic soil 16S rRNA gene clone libraries (Smith et al., 2000; Shivaji et al., 2004; Saul et al., 2005) and many have been cultured from Antarctic terrestrial environments and assigned to various genera, including *Sphingobacterium* (Wery et al., 2003; Shivaji et al., 2004; Saul et al., 2005), *Gelidibacter* (Smith et al., 2000), *Hymenobacter* (Hirsch et al., 1998; Saul et al., 2005) and *Chryseobacterium* (Wery et al., 2003). In soils, *Bacteroidetes*, recognised for their ability to degrade polymers, possibly play a role in decomposition (Buckley and Schmidt, 2001). In Antarctic soils polymers may be derived from wind-blown material from microbial mats or endolithic communities (Hopkins et al., 2005). Some *Bacteroidetes*, including those isolated in this study, produce extra-cellular enzymes such as lipases, proteases and phosphatases (Table 7; Hirsch et al., 1998; Wery et al., 2003) that have biotechnological potential due to their cold adaptation (Wery et al., 2003). In addition to providing substrate, the wind-blown material from microbial mats or endolithic communities could also serve as inocula for the soils. Ribotype C was most similar to clones from a cyanobacterium-dominated cryptoendolithic community from nearby Beacon sandstone (De La Torre et al., 2003). Similarly, ribotype B, being closely related to the marine bacteria *Gillisia mitskevichiae* and most prevalent in coastal soils of Marble Point, may be derived from marine sources via aeolian transport.

Eight distinct ribotypes belonged to the *Actinobacteria*, with five closely related to the genera *Arthrobacter*, *Friedmanniella* and *Rubrobacter*. The closest relatives of

the two (N and P) were most similar to clones from a cyanobacterium-dominated cryptoendolithic community from Beacon sandstone (De La Torre et al., 2003). *Arthrobacter* are ubiquitous in the Antarctic environment. They have been isolated from soils of the Dry Valleys, Marble Point (Table 4), Ross Island (Saul et al., 2005), and Schirmacher Oasis (Shivaji et al., 2004). The colonies of *Arthrobacter* are often highly pigmented, and our isolates were pigmented yellow, bright pink or cream (Table 7). *Friedmanniella* and *Rubrobacter* are less commonly reported but both genera have been detected in the McMurdo Dry Valleys environment. *Friedmanniella antarctica* DSM 11053^T was isolated from sandstone, from Linneaus Terrace in the Asgard Mountains (Schumann et al., 1997), whereas *Rubrobacter*, while not yet cultured from Antarctic soils, has been detected in clone libraries of soil from Ross Island (Saul et al., 2005) and in a cyanobacterium-dominated cryptoendolithic community (De la Torre et al., 2003). In soil, *Actinobacteria* such as *Arthrobacter* are common soil saprophytes recognised for their ability to metabolise a wide range of substrates including sugars and amino acids. Similarly, the *Arthrobacter* we isolated utilised a wider range of simple carbon sources than other bacteria characterised (Table 7). In these soils simple carbon compounds are released from cells following repeated freeze–thaw, including trehalose and glycerol stored in cells as compatible solutes, and *n*-acetylglucoasmine, a cell-wall component of many bacteria and fungi (Wynn-Williams, 1990).

Proteobacteria are common in soil although the prevalence of the alpha, beta, gamma and delta subdivisions differ (Zhou et al., 2003). In soils from the Wright Valley and Marble Point single ribotypes were assigned to each of the alpha, beta and gamma subdivisions. Although *Proteobacteria* have been detected in Antarctic soils before (Shivaji et al., 2004; Saul et al., 2005), the clones detected in this study were most closely related to uncultured bacteria from non-Antarctic sources, and their role in these soils is unknown. The dominance of alpha-*Proteobacteria* related to aerobic anoxygenic phototrophs in a cyanobacterium-dominated cryptoendolithic community in Beacon sandstone led to speculation that the organisms were contributing to primary production (de La Torre et al., 2003). Other roles *Proteobacteria* may play in these soils include the cycling of phosphorus. The closest cultured relative (99% similarity) of ribotype beta-*Proteobacteria* T is *Alcaligenes faecalis* WM2072, which is reported to oxidise reduced phosphorus compounds (Wilson and Metcalf, 2005). We cultured representatives of ribotype gamma-*Proteobacteria* U, whose closest known relative is a clone detected in deep sea sediments of the Ryukyu Trench (Li et al., 1999). Isolate U grew at 5 °C and exhibited a range of enzymatic activities including phosphatase and glycosidase, but the only carbon source used was trehalose (Table 7). The inability of this bacterium to tolerate salinity was surprising, given its prevalence in Bull Pass soil, which has very high electrical conductivity.

Library clones assigned to *Thermus-Deinococcus*, represented by three ribotypes (V, W, X) were prevalent in soils from the Wright Valley (Table 5, Fig. 3). The clones from this study were most closely related to uncultured *Deinococcus* bacterium clone FBP266 from a cyanobacterium-dominated cryptoendolithic community in Beacon sandstone (De La Torre et al., 2003), as were most clones from Ross Island (Saul et al., 2005). *Deinococcus* has been cultured from Antarctic soil from the Dry Valleys (Carpenter et al., 2000) and Ross Island (Saul et al., 2005). Although this genus is mostly recognised for radiation resistance, it is the ability of *Deinococcus* to tolerate desiccation, probably related to radiation resistance, which is likely to be of most significance for survival in the Dry Valleys environment (Carpenter et al., 2000). As well as being prevalent in Dry Valley soils, diverse *Deinococcus* spp. have recently been isolated from hot desert soil (Rainey et al., 2005), indicating that members of this genus may be dry soil specialists.

Acidobacterium spp. (ribotype Y), while common in control soil from Ross Island (Saul et al., 2005), was less prevalent in these soils from the Dry Valleys and Marble Point. Similarly, *Acidobacteria* were rare in cryptoendolithic communities in Beacon sandstone (de La Torre et al., 2003). Despite being widespread and abundant in soil, the role of *Acidobacteria* is unknown.

A single ribotype (AA) was most closely related to a clone from uranium-contaminated soil. A related cultured isolate is *Sporosarcina macmurdoensis* CMS 21w from a cyanobacterial mat in a pond on the McMurdo ice shelf (Reedy et al., 2003).

The primary producers of the soils of Victoria Land are *Cyanobacteria* and algae. In this study, phototrophs were represented by a single Cyanobacterial ribotype (AA). The ribotype was most closely related to an uncultured bacterium from a microbial mat in Lake Fryxell, of the Taylor Valley, and the closest cultured relative was *Leptolyngbya* sp. strain PCC9207 (Taton et al., 2003). As *Leptolyngbya* is dominant in all benthic mats of Victoria land (Adams et al. submitted), it is likely that it has been wind dispersed to Vanda soil from the extensive pinnacle mats that occur in nearby Lake Vanda or from surrounding ponds and streams (Hopkins et al., 2005).

Differences in distribution of the ribotypes may be explained in part by the differences in the soil moisture and temperature regimes of each site. Of note is the prevalence of *Actinobacteria* (in particular *Rubrobacter* (O) and ribotype P), *Deinococcus* (V, W, X), and *Bacteroidetes* (ribotypes I and H) in Wright Valley soils, and *Bacteroidetes* (B) in Marble Point soil. Bacteria such as *Rubrobacter* and *Deinococcus*, which prevail in the Wright Valley soils, may have a selective advantage if they are desiccation tolerant. We have shown previously that *Rubrobacter* clones from soils of the McMurdo Dry Valleys region cluster with those from hot desert soils, suggesting a relationship based on desiccation tolerance (Saul et al., 2005). For bacteria that do not produce resting

stages, such as spores or cysts, desiccation resistance has been attributed to accumulation of compatible solutes, membrane modification, production of extra-cellular polysaccharide (EPS) (Potts, 1994), or efficient repair of DNA damage (Mattimore and Battista, 1996). Desiccation resistance in *Deinococcus* has been linked to its DNA-repair abilities (Mattimore and Battista, 1996), whereas *Hymenobacter roseosalivarius* produces EPS (Hirsch et al., 1998). In addition, many *Hymenobacter* have been isolated from air or ice, indicating a possible relationship based on desiccation tolerance. The isolation of bacteria belonging to the clade containing ribotypes H, I, J and K would also be worth investigating for desiccation tolerance.

Cultured bacteria were represented in 8 of the 27 ribotypes and belonged to the divisions *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* and *Firmicutes*. While some of the isolates belong to ribotypes with closely related cultured strains (e.g., L, M, G, AA), the cultured members of ribotype U may represent a novel species or genus, being only distantly related to its closest cultured relatives (Table 5). That we did not isolate bacteria belonging to *Cyanobacteria* and *Acidobacterium* is not surprising. The culture conditions used in this study, R2A agar with incubation at 15 °C in the dark, would not allow growth of phototrophs such as *Cyanobacteria*. Furthermore, *Acidobacterium* spp. are not readily isolated from soil using traditional culturing methods.

While some genera within the *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* are readily isolated using R2A agar (Tables 6 and 7, Saul et al., 2005) different methods are required to isolate other genera, in particular those that contain the most dominant clones without cultured representatives (i.e. ribotypes B, I, T and O) detected in the soils. *Bacteroidetes*, for example, are reported to aerobically degrade polymers such as starch, gelatin, casein, xylan and Tween 80 (Hirsch et al., 1998), and the incorporation of these substrates in culture media could result in isolation of bacteria representative of ribotypes A, B, F, H, I and/or J. Similarly, pre-treatment of soil or soil dilutions with radiation, desiccation or multiple freeze–thaw cycles could enhance isolation of resistant microbes such as radiation-resistant *Deinococcus* or *Rubrobacter*.

The use of low nutrient media and long incubation times in particular has led to the isolation of novel bacteria from Antarctic sandstone or soil (Schumann et al., 1997; Hirsch et al., 1998). *Friedmanniella* for example was isolated from Antarctic sandstone on low nutrient media after incubation for 5 months at 9 °C under dim light (Schumann et al., 1997). Similarly, new isolates of the *Actinobacteria*, and *Proteobacteria* have been isolated from soil on low nutrient media following long-term incubation (Joseph et al., 2003). However, contrary to expectations, some Antarctic soil bacteria may be fastidious and require complex nutrients for growth. For example, O'Brien et al. (2004) reported that scree from beneath a rock face with endoliths from Battleship Promontory in the Dry Valleys had 10^9 CFU g^{-1} when enumerated on rich Tryptic Soy Agar.

In conclusion, dominance of a few ribotypes is a feature of the bacterial communities of soils of Marble Point and the Wright Valley; however, the dominant ribotypes do vary between locations. Dominance in bacterial communities may be due to their enhanced ability to utilise limited resources and to the survival of the better adapted species. A key difference between the sites was the availability of water. Soils from the coast at Marble Point had the most diverse bacterial community and were moister during summer, whereas the drier soils of the Wright Valley had a less diverse bacterial community. The prevalence of some bacterial groups in Wright Valley soil, such as *Deinococcus* and *Rubrobacter*, could be due to their desiccation tolerance. Some clones or bacteria in the soils were most closely related to those from nearby Antarctic environments including microbial mats and a cyanobacterium-dominated cryptoendolithic community, indicating that the soil may have been inoculated from these sources by wind dispersal. Some of the dominant bacterial ribotypes (A, F, H, I, J, K, U and Y) appear to be novel, being only distantly related (<95%) to cultured bacteria or clones in GenBank.

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