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Culturable aerobic heterotrophic bacteria from high altitude, high latitude soil of La Gorce Mountains (86°30'S, 147°W), Antarctica

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Abstract: Eleven aerobic heterotrophic bacteria isolated from soil samples taken at *c.* 1800 m altitude from La Gorce Mountains, Antarctica, were characterized in terms of carbon source utilization, enzymatic activities and tolerance to environmental stressors. The bacteria typically formed pigmented colonies on agar plates and were initially observed on media designed for isolation of algae and cyanobacteria. The bacteria were purified and assigned to the *Actinobacteria*, *Bacteroidetes* or *Proteobacteria* divisions following 16S rRNA gene sequence analysis. While some of the isolates are most likely to belong to the genera *Arthrobacter* or *Brevundimonas*, others may belong to novel genera or species. The Gram-positive *Actinobacteria* used the widest range of carbon sources for growth. *Brevundimonas* P7 produced lipases, phosphatases, proteases and glycosyl-hydrolases. The Gram-positive bacteria were more tolerant to freeze-thaw than the Gram-negative isolates. No isolates survived more than 10 minutes ultraviolet irradiation. All isolates were unaffected by 24 h desiccation. This study adds to knowledge of the bacterial diversity of soils from high altitude (1800 m), high latitude (86°30'S) locations within Antarctica.

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Introduction

The prevailing low temperatures, low humidity, freeze-thaw cycles, and salinity of soils of continental Antarctica combine to create a harsh environment for plant and animal life (Campbell & Claridge 1987). Only a few animals and plants have managed to colonize and survive in these conditions. Bacteria, however, are distributed throughout Antarctic soils, with higher numbers detected in coastal soils than in arid inland soils of the McMurdo Dry Valleys (Vishniac 1993).

Early investigations of bacteria in Antarctic soils have focused on abundance and diversity and the influence of climatic conditions (reviewed by Vishniac 1993). Typically, the bacteria described were aerobic heterotrophs forming pigmented colonies on solid media. These bacteria were assigned to known genera including *Arthrobacter*, *Micrococcus*, *Bacillus* and *Pseudomonas*. Coryneforms were typically dominant, whereas *Pseudomonas* was rare (Johnson *et al*. 1978). These studies led to the conclusion that bacteria from Antarctic soils may be cosmopolitan and similar to those found in soils worldwide. In recent years the increased use of molecular tools, such as 16SrRNA gene sequencing and phylogenetic analysis, has provided new insights into the genetic affinities and diversity of Antarctic bacteria (Vincent 2000). Consequently, novel bacteria have been described from Antarctic soils, for example see Hirsch *et al*. (1998), Mevs *et al*. (2000) and Wery *et al*. (2003).

Most studies of bacterial diversity in soils have focused on inland desert or coastal locations (Vishniac 1993). Investigations of bacterial diversity of high altitude, high latitude locations are sparse. For soil collected from La Gorce Mountains (1800 m altitude and 86°30'S) Claridge *et al*. (1971) reported no culturable organisms in two samples, whereas Cameron reports that soil from near a pond yielded low numbers of bacteria that were identified as *Arthrobacter* (Cameron 1972). *Arthrobacter* and *Corynebacterium* have also been isolated from soil from Mount Howe (2800 m altitude and 87°12'S); (Cameron *et al*. 1971). Broady & Weinstein (1998) visited La Gorce Mountains to survey lichens, eucaryotic algae and cyanobacteria, and fungi, and subsequently Lawley *et al*. (2004) used molecular tools to investigate the *in situ* eucaryote diversity in soil from La Gorce Mountains and other sites.

In this paper we describe eleven heterotrophic bacteria from soil of La Gorce Mountains. The bacteria were originally isolated from soil inoculated onto agarised mineral salts culture medium designed for the growth of eucaryotic algae and cyanobacteria (Broady & Weinstein 1998). The bacteria were identified to genus level by 16S rRNA gene sequence analysis and characterized for carbon sources that stimulate growth, enzymatic activities and tolerance to environmental stressors.

Materials and methods

Source of isolates

Soil samples from glacial moraines at *c.* 1800 m altitude in La Gorce Mountains were collected using aseptic precautions by P.A. Broady in January 1997 (Broady & Weinstein 1998). The bacterial isolates used in this study were initially observed as colonies growing on agarised BG-11 medium (Rippka *et al.* 1979) inoculated with soil from locations A and B at La Gorce Mountains. The origianl intention of these cultures was for the isolation of eucaryotic algae and cyanobacteria (Broady & Weinstein 1998). Colonies of approximately 25 aerobic heterotrophic bacteria were subsequently removed from the BG-11 isolation plates that did not yield photosynthetic micro-organisms, transferred to R2A agar plates using a sterile loop (Difco) and incubated at 4°C in the dark. The cultures were purified by continual subculture on R2A plates, and 11 isolates that maintained viability were characterized by cell morphology, Gram stain and molecular analysis. As all the isolates grew faster at 15°C than 4°C, they were routinely maintained at 15°C on R2A plates. The isolates have been deposited in the International Collection of Microorganisms from Plants (ICMP, c/o Landcare Research, Private Bag 92170, Auckland, New Zealand).

Chemical and physical analyses of soils

Soil from locations A and B described in Broady & Weinstein (1998) were pooled to give < 8 g. The soil was lightly crushed and the < 4 mm fraction was analysed for pH, total carbon, nitrogen and phosphorus, bioavailable phosphorus, electrical conductivity, total soluble salts, and exchangeable cations using standard methods (Blakemore *et al*. 1987). The ratings for soil chemical properties follow Blakemore (*et al*. 1987) and are for soils in general.

DNA extraction, PCR amplification and sequencing of amplified 16S rDNA

Genomic DNA was extracted from approximately 0.1 g of bacterial colonies recovered from the surface of an R2A agar plate with a sterile pipette tip as described previously (Foght *et al*. 2004). Bacterial 16S rRNA genes were amplified by PCR using the oligonucleotide primers PB36 (5'-AGRGTTTGATCMTGGCTCAG-3') and PB38 (5'-GKTACCTTGTTACGACTT-3') corresponding to *E. coli* positions 8–27 and 1492–1509, respectively (numbering as Brosius *et al*. 1981). PCR was carried out as described previously (Foght *et al*. 2004). PCR products were purified using a High Pure PCR Purification Kit (Roche). All sequencing was on a 3100 Genetic Analyser capillary electrophoresis sequencer (Applied Biosystems). Near full-length 16Sr rRNA gene sequences were assembled in Auto Assembler (Applied Biosystems) and compared with known sequences in GenBank (http://www.ncbi.nlm.nih.gov) using the BLAST search tool.

Phylogenetic methods

Where a full phylogenetic method was performed, 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 which helped to break long branches. All phylogenetic analyses were performed using PAUP* (Swofford 2002). Because of the number of taxa within the *Actinobacteria* 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. For the *Proteobacteria* trees, a Neighbour Joining starting tree was refined under the criterion of Maximum Likelihood with the parameters in the evolutionary model estimated from the starting tree.

Utilization of carbon sources and enzyme activities

The isolates were screened for their ability to metabolize various compounds as a carbon source. These carbon compounds were selected to represent those most likely to be available as exudates of algae/cyanobacteria (Siebert & Hirsch 1988), which are the primary producers in soils of La Gorce Mountains. Metabolism of 5 mM acetate, *n*-acetylglucosamine, adonitol, d-arabitol, citrate, gluconate, glucose, glutamate, lactate, mannitol, mannose, oxalate, pyruvate, ribose, succinate and trehalose was determined on Bushnell Haas minimal medium (Difco) solidified with 1.5% purified agar (Difco) and amended with yeast extract (0.005 g L^{-1}) as a source of growth factors. The carbon sources were sterilized separately and added to sterile medium. Plates were inoculated with 4 x 10 ì L drops of cell suspensions prepared using cultures grown on R2A plates for less than 4 weeks. Cell suspensions were prepared by harvesting cells from the plates and resuspending the pellets to *c.* 10^9 CFU mL^{-1} in 10 mM phosphate buffer (pH 7).

Plates were incubated at 15°C for up to six weeks. Growth was compared with that on minimal agar plates prepared without additional carbon source.

Oxidase and catalase tests were determined on cultures grown on R2A using standard methods (Gerhardt 1994). Hydrolysis of DNA was determined on DNase Agar (Difco), starch and gelatin on R2A agar amended with 0.3% (w/v) soluble starch and 0.4% (w/v) gelatin respectively as described by Hirsch *et al*. (1998) and lipase activity on R2A agar plates supplemented with 0.5% (w/v) Tween 80 and 0.01% (w/v) $CaCl_2.H_2O$ (Gerhardt 1994). All plates were inoculated with cell suspensions and incubated at 15°C for

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Table I. Properties of soil samples from La Gorce Mountains.

pH (water)	Total C (%)	Total N (%)	Total P (mg kg^{-1})	Bioavailable P (mg kg^{-1})	EC (mS cm^{-1})	Total soluble salts (%)	Exchangeable Ca	Mg	K	Na
							cmol(+)kg^{-1}			
Site A - mineral fines, La Gorce cirque, moraine on east side										
5.1	0.09	0.008	341	0.5	0.03	0.01	1.59	0.13	0.00	0.00
5.7	0.11	0.009	261	0.5	0.05	0.02	2.26	0.34	0.02	0.02
Site B - mineral fines, La Gorce cirque, moraine on west side										
4.8	0.07	0.004	448	0.6	0.03	0.01	0.91	0.13	0.00	0.00
4.7	0.04	0.002	435	0.1	0.03	0.01	1.62	0.15	0.00	0.00

up to four weeks. In addition, APIZYM test strips (Biomerieux, Lyon, France) were used to screen the isolates for a range of hydrolytic enzymatic activities. The strains were grown on R2A and resuspended in solution provided by manufacturers. After inoculation, the APIZYM test strips were incubated for 48 h at 15°C before the results were recorded.

Tolerance to environmental stressors

Growth at a range of temperatures was determined on R2A plates incubated at 0, 4, 15, 20, 25 or 30°C. Tolerance to pH (5, 6, 7, 8, 9.5, 11 and 12) was determined on R2A agar plates supplemented with 0.05 g L^{-1} each of KH_2PO_4 and $Na_2HPO_4.2H_2O$ and the pH adjusted from pH 5 to pH 12 as required; the actual pH of the cooled medium was remeasured aseptically after heat sterilization. Salt tolerance was determined using R2A plates amended with NaCl (0, 1, 2, 4 and 6% w/v). As described above, agar plates were inoculated with 4 x 10 µL drops of cell suspensions prepared using cultures grown on R2A. All plates were incubated at 15°C for up to six weeks.

Resistance of the cells to UV irradiation was determined by the method of Baraniecki *et al*. (2002). Cells were serially diluted in phosphate buffer (pH7), plated in triplicate on R2A plates, treated with UV for 0, 2, 5 and 10 minutes and incubated at 15°C for four weeks. Tolerance to freeze-thaw was determined by dispensing replicate 0.1 mL portions of cell suspensions into sterile microcentrifuge tubes containing 1 g of sterile acid washed sand (Unilab) and frozen at -70°C. For each thaw cycle, the tubes were placed at 4°C for 1 hour and a sample of each strain was selected randomly. The remaining aliquots were re-frozen, while the selected samples were serially diluted in

Table II. Taxonomic affiliations of bacteria isolated from soils of La Gorce Mountains determined by sequencing 16S rRNA genes.

Bacterial group	Source of isolates Site A	Site B	Colony colour	Accession #	Division	16S r DNA analysis Phylogenetic nearest taxon (GenBank accession #; source % identity)
Gram-positive rods/coccibacilli						
P1, P2	X		Red Circular dry	DQ351726, DQ351727	*Actinobacteria*	Uncultured actinobacterium clone Cli20 (AF529327, PCE contaminated site, 98%)
P19		X	Yellow Circular Smooth	DQ351734	*Actinobacteria*	*Arthrobacter agilis* LV7 (AF134184, Antarctic microbial mat, 100%)
P6, P20		X	Orange Smooth Circular	DQ351730, DQ351735	*Actinobacteria*	Uncultured bacterium clone AKAU3869, DQ125756, uranium contaminated soil, 97%)
P23	X		Orange Smooth Circular	DQ351736	*Actinobacteria*	*Nocardia arthritidis*, AB0108781, pathogen, 94%)
Gram-negative rods						
P3	X		Pink-red Shiny Tight	DQ351728	*Bacteroidetes*	Uncultured bacterium clone ARKCRY2 (AY198110, Arctic sea ice, 95%)
P4, P14	X	X	White/cream Translucent Smooth Circular	DQ351729, DQ351733	*β-Proteobacteria*	Uncultured bacterium clone 4312H (AY571835, Antarctic soil, 98%)
P7, P8		X	Yellow Circular Smooth	DQ351731, DQ351732	*α-Proteobacteria*	Glacier bacterium FXI13 (AY315163, ice Fox Glacier, 99%)

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phosphate buffer (pH 7) and plated in triplicate onto R2A plates to determine viability. All plates were incubated at 15°C for up to six weeks. Tolerance of P3 to freeze-thaw and UV was not determined because the cells do not form even cell suspensions nor do they form colonies when diluted and spread on agar plates.

Desiccation tolerance was determined using a filter membrane technique as described by Baraniecki *et al.* (2002).

Results

Soil analyses

The soil was coarse-textured sand, moderate to strongly acid and with very low levels of total carbon and nitrogen (Table I); some of the nitrogen may have been nitrate which was present at high levels in water (425.8–1900.5 mg m^{-3} NO_3–N) from nearby ponds (Broady & Weinstein 1998). Total P levels were low to medium and bioavailable P was very low. Predominant cations were Ca^{2+} and Mg^{2+}.

Characterization of bacterial isolates

Eleven bacterial isolates capable of sustained growth on R2A were characterized (Table II). The isolates included both Gram-positive and Gram-negative organisms and were rods or coccibacilli with some cultures forming filaments. None of the isolates were able to grow in liquid R2A broth. Most of the isolates formed pigmented colonies on R2A agar plates.

The 11 bacterial isolates were assigned to seven taxonomic groups; six belonged to the *Actinobacteria*, one to the *Bacteroidetes* group, and two to each of the α- and β-*Proteobacteria* (Table II; Figs 1 & 2). Some of the bacteria

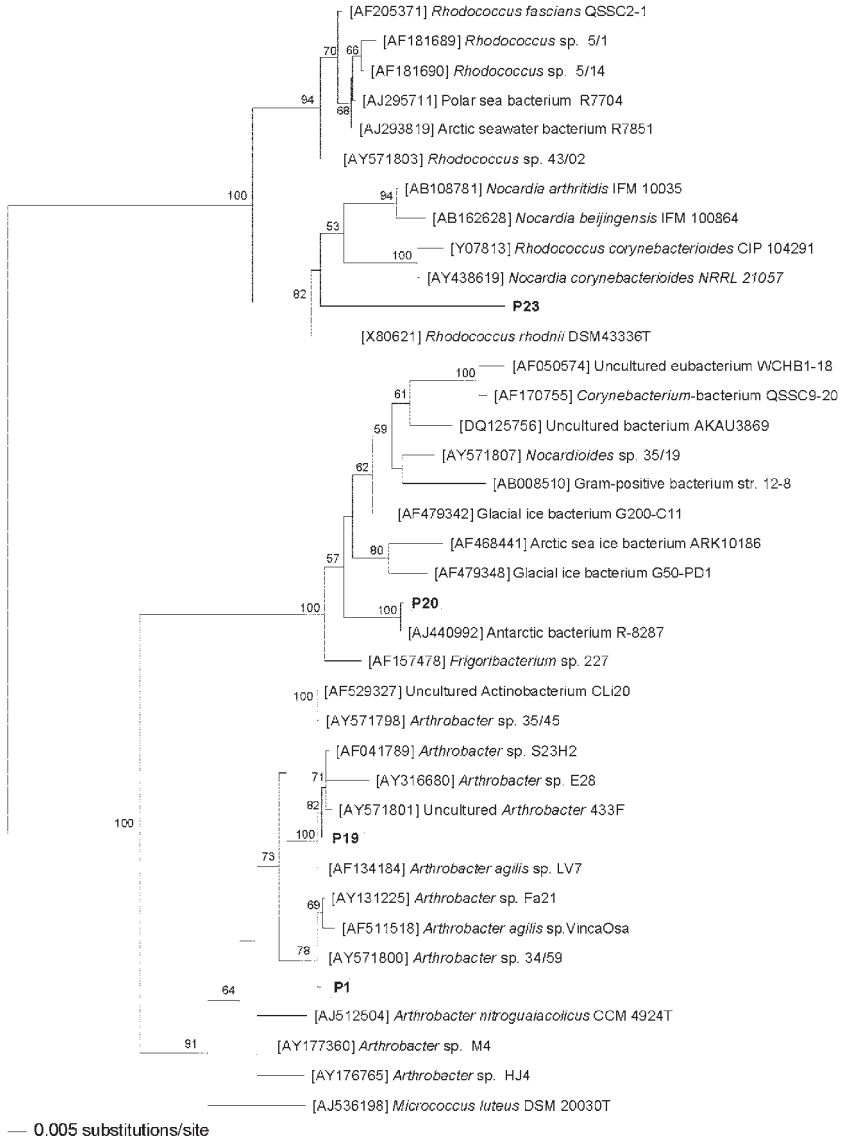

Fig. 1. Mid-point rooted Neighbour Joining tree of the *Actinobacteria* isolated from soil from La Gorce Mountains. The tree was constructed from 1480 sites of the aligned 16S rRNA genes. Numbers above the branches are bootstrap values estimated from 1000 Neighbour Joining re-sampling replicates. Where a clade has no associated number the bootstrap values are below 50%.

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had identical 16SrDNA sequence. They were P1 and P2, P6 and P20, P4 and P14, and P7 and P8. Of the six isolates assigned to *Actinobacteria*, BLAST searches and subsequent phylogenetic analysis indicated that P1, P2 and P19 were *Arthrobacter* spp. and most closely related to *A. agilis*. P20 and P6 grouped with the genus *Frigoribacterium* whereas P23 is distantly related to *Nocardia* and *Rhodococcus*. The Gram-negative isolate P3, which produced extracellular polysaccharide (EPS), was assigned to the *Bacteroidetes* group and was most closely related to a clone from Arctic sea ice (Table II). Of the *Proteobacteria*, P4 and P14 belonged to the β-*Proteobacteria* (Fig. 2), whereas P7 and P8 clustered with *Brevundimonas*, a member of the α-*Proteobacteria* (Fig. 2). Seven isolates representative of the bacterial groups were chosen for further characterization.

Carbon sources and growth stimulation

The isolates varied in their ability to metabolize the various carbon sources (Table III). In general, growth of Gram-positive isolates was more readily stimulated. For example, *Arthrobacter* P1 and P19 were stimulated by 12 and 7 of the carbon sources respectively, whereas none of the carbon sources stimulated growth of two of the Gram-negative isolates, P3 and P7. Glutamate was the most widely metabolized carbon compound. It was utilized by 5 of the isolates, whereas d-arabitol, adonitol and oxalate failed to stimulate the growth of any isolates.

All isolates were catalase positive but varied with respect to oxidase (Table III). All isolates were positive for lipolytic activity (esterase lipase - C8), with P3, P7 and P19 also hydrolysing Tween 80. *Brevundimonas* P7, P3 and P4 were most active and exhibited proteolytic and phosphatase activities. Four isolates showed glycosyl-hydrolase activity. None of the isolates hydrolysed DNA.

Environmental tolerance

All isolates were psychotolerant as they grew at 4 and 15°C. All except for P4 and P23 grew at 0°C. Only P19 and P4 grew at 25°C but not 30°C (Table III). None of the isolates grew at pH 5. All grew at pH 8 except for P3. *Arthrobacter* P1 and P19 were most tolerant to alkaline conditions growing at pH 11 but not 12 (Table III). *Arthrobacter* P19 was most tolerant to salt. It grew on R2A plates with 4% (w/v) NaCl but not 6%, whereas all Gram-negative isolates were inhibited by 1% NaCl (Table III).

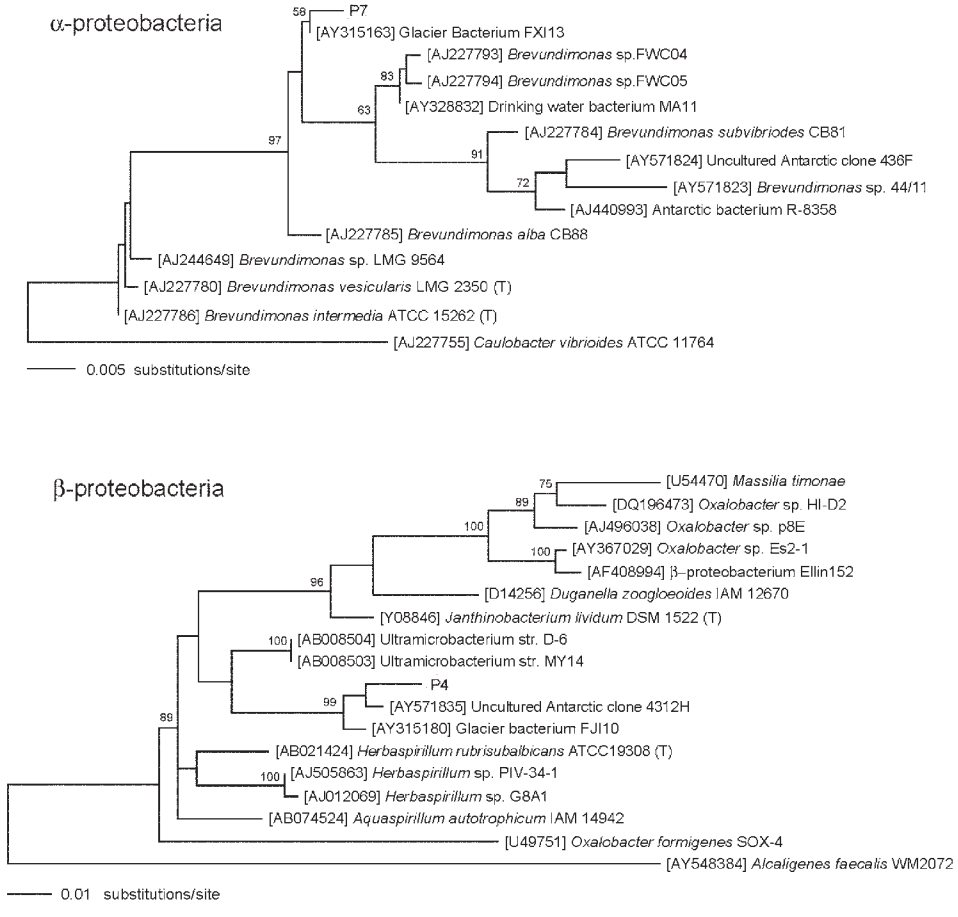

Fig. 2. Rooted Maximum Likelihood trees of the *Proteobacteria* isolated from soil from La Gorce Mountains. The tree was constructed from 1408 (alpha) and 1505 (beta) sites of the aligned 16S rRNA genes. Numbers above the branches are bootstrap values estimated from 1000 Maximum Parsimony re-sampling replicates. Where a clade has no associated number the bootstrap values are below 50%.

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Table III. Growth charactersitics of bacteria isolated from soil of La Gorce Mountains, Antarctica.

Bacterial division	*Actinobacteria*				*Bacteroidetes*	*Proteobacteria*	
Bacterial ID	P1	P19	P20	P23	P3	P4	P7
Carbon sources that enhanced growth:							
Glucose	+[1]	+	+	+	-	-	-
Trehalose	+	+	+	+	-	-	-
Ribose	+	+	+	+	-	-	-
n-Acetylglucosamine	+	+	-	-	-	-	-
Mannose	+	+	-	+	-	-	-
Mannitol	+	-	-	-	-	-	-
Gluconate	+	+	+	-	-	-	-
Citrate	-[1]	-	-	-	-	+	-
Pyruvate	+	-	+	+	-	-	-
Succinate	+	-	-	-	-	-	-
Acetate	+	-	+	-	-	+	-
Lactate	+	-	+	+	-	+	-
Glutamate	+	+	+	+	-	+	-
Enzymatic activities[2]							
Oxidase	-	+	-	-	-	-	+
Catalase	+	+	+	+	+	+	+
Hydrolysis of: [2]							
Tween 80	-	+		-	+	-	+
Starch	-	+	+	+	-	-	-
Gelatin	-	-	-	-	+	-	+
API Zym test: [3]							
Phosphatase	+	+	-	-	+++	+++	++
Lipase	+	+	+	+	+	++	++
Protease	+	+	++	+	++	+	+++
Glycosidase	+	+	-	-	-	+	+
Temperature range that supported growth (°C)	0–20	0–25	0–15	4–15	0–20	4–25	0–20
pH range that supported growth	7–11	7–11	7–8	7–8	6–7	6–8	7–8
Salinity range that supported growth (%NaCl w/v)	0–1	0–4	0–2	0–2	0	0	0

[1]Where "+" means growth enhanced and "-" means no growth enhanced.
[2]Enzymatic activities determined on agar plates and scored where "+" means positive and "-" means minus
[3]Enzymatic activities as revealed by APIZYM tests, scored on an arbitrary scale from "+" increasing to "+++".

The viability of all isolates was reduced following exposure to 2 min UV, with P20 most tolerant (Table IV). By 10 min, less than 1% of cells remained viable. Gram-positive isolates were more tolerant of freeze-thaw cycles than Gram-negative isolates (Table V). *Arthrobacter* P1 was unaffected by 10 freeze-thaw cycles, whereas < 1% of P4 cells were viable following one cycle. P19 and P23 were similarly affected, with > 30% cells remaining viable after 10 freeze-thaw cycles, whereas < 5% of P7 remained viable.

All isolates were unaffected by 24 h desiccation (data not shown). The desiccation tolerance of P4 (or P14) was not determined because the bacterium was unable to grow on a membrane filter.

Discussion

The bacterial isolates characterized in this study from high altitude, high latitude soil of La Gorce Mountains were assigned to *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*.

Actinobacteria are metabolically diverse soil saprophytes commonly isolated from Antarctic soils. Within this division the genera *Arthrobacter*, *Micrococcus*, and *Rhodoccocus* are considered ubiquitous in Antarctic soils (Smith *et al*. 2000). Three of the isolates, the red pigmented P1 and P2, and the yellow pigmented strain P19, were identified as *Arthrobacter* and clustered with two different

Table IV. Effect of UV treatment time (mins) on percent survival of cell suspensions of bacterial isolates from soils of La Gorce Mountains.

Bacterial division	*Actinobacteria*				*Proteobacteria*	
Isolate	P1	P19	P23	P20	P4	P7
Time (mins)	Percent viability					
0	100 ± 21.5	100 ± 6.1	100 ± 12.8	100 ± 6.8	100 ± 12.8	100 ± 17.2
2	17.4 ± 2.9	22.0 ± 2.9	21.3 ± 7.5	83.1 ± 8.7	22.8 ± 2.8	< 1
5	7.6 ± 1.8	1.5 ± 0.1	8.6 ± 3.6	3.7 ± 1.8	2.2 ± 0.4	< 1
10	< 1	< 1	< 1	< 1	< 1	< 1

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Table V. Effect of number of freeze-thaw cycles on the percent survival of bacterial isolates from La Gorce Mountains.

Bacterial division	*Actinobacteria*				*Proteobacteria*	
Isolate ID	P1	P19	P20	P23	P4	P7
Number of cycles	Percent viability					
0	100 ± 15.2	100 ± 4.9	100 ± 19.9	100 ± 3.7	100 ± 5.2	100 ± 10.0
2	116.1 ± 13.9	105.7 ± 6.8	98.8 ± 15.2	79 ± 12.7	< 1	45 ± 5.9
5	118.7 ± 17.6	63.8 ± 8.4	25.6 ± 7.2	76.7 ± 11.3	< 1	53.1 ± 4.5
10	117.4 ± 18.9	43.8 ± 5.9	2.9 ± 1.1	33.3 ± 3.9	< 1	4.4 ± 1.1

strains of *Arthrobacter agilis* (Fig. 1). *Arthrobacter* spp. were isolated from many different Antarctic sources including soil (Cameron *et al*. 1971, Siebert & Hirsch 1988, Wery *et al*. 2003, Saul *et al*. 2005) and microbial mats (Loveland-Curtze *et al*. 1999, Reddy *et al*. 2000, Brambilla *et al*. 2001). *Arthrobacter* forming beige or orange colonies was isolated from soil from La Gorce Mountains collected from near a pond (Cameron *et al*. 1971). The red pigmented *Arthrobacter* strains P1 and P2 appear similar to the *Micrococcus* spp. from rock or soil samples of the McMurdo Valleys, described by Siebert & Hirsch (1988). Like P1, the *Micrococcus* species were red pigmented, Gram-positive coccibacilli, catalase positive and tolerant to alkaline pH. However, P1 was negative for phosphatase activity, utilized a wider range of carbon sources and was less salt tolerant. P19 was identical to the psychrophile *Arthrobacter agilis* LV7 (Table II, Fig. 1) and they share many characteristics. Both strains form yellow pigmented circular convex colonies, grow at temperatures of less than 5°C but not at 30°C, tolerate 3.5% NaCl, and possess β-galactosidase activity (Loveland-Curtze *et al*. 1999).

P6 and P20 grouped with a cluster (Fig. 1) containing *Frigoribacterium* 227 and many isolates or clones from cold environments, including *Frigoribacterium* sp. 34/19 isolated from Antarctic soil from Ross Island (Saul *et al*. 2005). The type species *Frigoribacterium faeni*, including *Frigoribacterium* 227, which belongs to genomovar 2, was isolated from dust in a cattle barn in Finland (Kämpfer *et al*. 2000). Like the type species, P20 was Gram-positive, with irregular shaped rods that do not form mycelia, and grow at 4°C. Catalase is produced but not oxidase, and sugars are utilized as carbon sources. P23 appears to be a psychrophile (Table III) and being only distantly related to *Nocardia* and *Rhodococcus* (Fig. 1) is most probably a new species or possibily a new genus.

Members of the *Bacteroidetes* group are also frequently isolated from Antarctic soil. Phylogenetic analysis indicates that the P3 groups with the genus *Hymenobacter* (Aislabie *et al.* in press). *Hymenobacter* was first described from soils of Linneaus Terrace (1600 m, Asgard Range, Antarctica) (Hirsch *et al*. 1998). *Hymenobacter* has subsequently been isolated from soil from Ross Island (Saul *et al*. 2005), and was detected in clones from air over the Antarctic Peninsula (Hughes *et al*. 2004). As in this study, the *Hymenobacter* strains isolated by Hirsch *et al*. (1998) were originally isolated under low light intensities. Similar to the isolates of Hirsch *et al*. (1998), P3 formed red- to pink-pigmented colonies; however, they were contained and tight rather than spreading and thin. P3 also differed biochemically from the strains described by Hirsch *et al*. (1998). It did not grow on any of the tested carbon sources, or above 25°C. However, P3 does aerobically degrade the polymers gelatin and Tween 80. The *Hymenobacter* strains isolated by Hirsch *et al*. (1998) were assigned to a new species *Hymenobacter roseosalivarius*. P3 may also belong to a new species.

The isolates P4 and P14 and P7 and P8 were assigned to the â-*Proteobacteria* and the α-*Proteobacteria* respectively (Table II, Fig. 2). P4 and P14 were most similar to clone 4312H from soil from Scott Base, Ross Island (Saul *et al*. 2005), which clustered with bacterium FJI10 isolated from ice from Franz Joseph Glacier, New Zealand (Foght *et al*. 2004). P4 is assigned to the family Oxalobacteriaceae but has no close relatives identified to genus or species level (Fig. 2). A relative of P4, *Oxalobacter* sp. p8E was isolated from soil from Cape Evans, Antarctica (Wery *et al*. 2003). P7 and P8 were most similar to bacterium FXI13 isolated from ice from Fox Glacier, New Zealand (Foght *et al*. 2004), and grouped with *Brevundimonas*. *Brevundimonas* has been isolated from Antarctic soil from Cape Evans (Wery *et al*. 2003), Schirmacher Oasis (Shivaji *et al*. 2004), and Scott Base (Saul *et al*. 2005). P7 shares similar characteristics with *Brevundimonas* P8H (Wery *et al*. 2003). Both strains form yellow colonies, grow at 4°C but not 37°C, are oxidase and catalase positive, and exhibit proteolytic, lipolytic and phosphatase activities and little glycosyl-hydrolase activity.

For bacteria to survive and grow in La Gorce Mountain soils they must be tolerant of *in situ* conditions. The bacteria described in this study were isolated from mineral soils from amongst boulders on ice-cored moraines. The soil depth was a few millimetres to *c.* 3 cm thick. During sampling between 3 and 20 January 1997 the mean air temperature was -14.1°C, but the temperature of moist soil in full sun ranged from +1 to +7°C, and the maximum temperature measured for dry soil was 12.6°C (Broady & Weinstein 1998). During fine summer weather it is likely soil temperatures show diurnal variations correlated with incoming solar radiation, as has been observed for other soils in the Ross Sea region (Balks *et al*. 2002). Hence, conditions for bacterial growth and activity in these soils

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may occur during summer when soils are moist and temperatures are > 0°C. The bacteria described are all cold tolerant and grew at 4°C, and 15°C. Furthermore, five out of seven grew at 0°C but the lowest temperature for growth was not determined. As the soil salts are low (Table I) the bacteria are unlikely to be under osmotic pressure, except when soil moistures are low. This is reflected in the inability of most of the isolates to tolerate > 1% NaCl (w/v) (Table III).

The diurnal variations in soil temperature result in multiple freeze-thaw events during summer (Balks *et al*. 2002). The freeze-thaw tolerance of all the isolates, except P4, was greater than that of *Escherichia coli* ATCC 25922 determined under similar conditions (Baraniecki *et al*. 2002). Of note, the viability of strain P1 was not affected by ten freeze-thaw cycles (Table V). The lack of freeze-thaw tolerance of P4 may be due to changes in cell wall chemistry upon growth and storage in the laboratory. The morphology of colonies of P4 and P14 was observed to change during continual culture in the laboratory and it is therefore possible that the changed morphology was due to a mutation of the cell wall that lead to loss of freeze-thaw tolerance. Originally P4 and P14 formed hard dull colonies that were difficult to transfer from one agar plate to another. During subculture the cultures changed from a hard, dull, colony type to a mix with moist colonies, and eventually only the moist colony type was produced. Alternatively, P4 may have entered a viable but non-culturable state following freeze-thaw.

When the soils are dry or frozen the bacteria will be under water stress and will need to be desiccation tolerant. Close relatives of some of the isolates (e.g. P6 and P20, P3) were from air or ice, indicating a relationship based on desiccation tolerance. All the isolates tolerated at least 24 h in the desiccator, similar to *Deinococcus radiophilus*, but were considerably more tolerant than *E. coli* (Hugenholtz *et al*. 1995). Desiccation tolerance mechanisms in bacteria that do not produce cysts or spores have been attributed to accumulation of compatible solutes, alteration of cell membranes, production of extracellular polysaccharide (EPS) (Potts 1994), and efficient DNA repair (Mattimore & Battista 1996). The ability of P3 to tolerate desiccation may be due to production of EPS, which is reported to be a common feature of Antarctic desert microbes (Wynn-Williams 1990). To our knowledge, however, investigations of desiccation tolerance mechanisms of Antarctic heterotrophic bacteria similar to those we have isolated have not been described, despite the need for Antarctic soil bacteria to survive from one soil moistening event to the next.

Additional stressors for growth and activity of heterotrophic bacteria include low nutrients and acidic pH. At the soil sampling sites in the La Gorce Mountains, carbon could be derived from cyanobacteria which form thin benthic mats in ice covered ponds, and from the usually sparse, microscopic populations of cyanobacteria and eucaryotic algae in the soils (Broady & Weinstein 1998). Microscopic fragments of mats were found in almost half the 45 soil samples from Site B suggesting that benthic mats are widely dispersed, probably by wind. In southern Victoria Land Dry Valleys, carbon in the moraines has been sourced to freeze-dried cyanobacterial mats from adjacent ponds (Hopkins *et al*. 2005), whereas nitrogen as nitrate derives primarily from atmospheric deposition (Wada *et al*. 1981). Some of the bacteria (P3, P4 and P7) produce exoenzymes, which would contribute *in situ* to degradation of polymers, while others (P1, P19, P20) utilize simple carbon compounds such as organic acids and sugars (Table III). These would be released during degradation of polymers and as a consequence of freeze-thaw processes (Wynn-Williams 1990). Surprisingly, none of the isolates grew at pH 5, despite the bulk pH of the soil being measured at 4.7–5.7. It is possible that the micro-sites where these bacteria are active are less acidic than indicated by measurement of bulk soil pH. Alternatively, the bacteria described in this manuscript may exist in the soil as inactive propagules. Heterotrophic bacteria can be introduced into these soils with windblown microbial mats from nearby lakes or ponds, or with wind from even further afield (Vincent 2000). Interestingly, the *Arthrobacter* strains P1 and P19 were tolerant of alkaline conditions (Table III) more typical of the pH of coastal soils in the Ross Sea region, whereas the *Proteobacteria* P4 and P8 were most closely related to bacteria from New Zealand glacier ice (Fig. 2).

The heterotrophic bacteria described here from high altitude, high latitude soils of La Gorce Mountains have characteristics that enable them to survive very harsh Antarctic conditions *in situ*, including tolerance of cold, desiccation and freeze-thaw. To date there have been few investigations of the underlying molecular mechanisms for these tolerances. These bacteria are worthy candidates for such investigations. While some of the isolates most likely belong to genera commonly reported in Antarctic soils (eg. *Arthrobacter* and *Brevibacterium*), others may belong to novel genera or species.

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