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ORIGINAL PAPER

Cold-active halophilic bacteria from the ice-sealed Lake Vida, Antarctica

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Abstract Lake Vida is a large, permanently ice-covered lake in the Victoria Valley of the McMurdo Dry Valleys, Antarctica and is unique among Dry Valley lakes because it is ice-sealed, with an ice-cover of nearly 19 m. Enrichment cultures of melt-water from Lake Vida 15.9 m ice yielded five pure cultures of aerobic, heterotrophic bacteria. Of these, one strain grew at −8°C and the four others at −4°C. All isolates were either halotolerant or halophilic, with two strains capable of growth at 15% NaCl. Phylogenetic analysis revealed the Lake Vida isolates to be *Gammaproteobacteria*, related to species of *Psychrobacter* and *Marinobacter*. This is the first report of pure cultures of bacteria from Lake Vida, and the isolates displayed a phenotype consistent with life in a cold hypersaline environment.

Keywords Antarctica · Lake Vida · Psychrophiles · *Psychrobacter* · *Marinobacter*

Introduction

The McMurdo Dry Valleys are one of the few ice-free regions in Antarctica. As polar deserts, the Dry Valleys experience high winds and extremely cold average air temperatures (Spigel and Priscu 1998; Gordon et al. 2000). Several lakes have formed in the Dry Valleys from glacial erosion and are some of Earth's southernmost biologic oases (Matsumoto 1993; Priscu et al. 1998). Dry Valley lakes are unique in many ways, in particular their constantly cold temperatures, permanent ice-cover, absence of wind mixing, and lack of higher organisms. The water columns of Dry Valley lakes are therefore extremely stable and the ecosystems entirely microbial (Fountain et al. 1999; Priscu et al. 1999).

Although Dry Valley lakes are geographically relatively close to one another, their limnology differs dramatically. In this regard, Lakes Fryxell, Hoare, and Bonney, the major lakes in the Taylor Valley, each have a unique geochemistry and have been the best studied of all Antarctic lakes (Priscu 1998). Ice-covers on Taylor Valley lakes typically range from 3–7 m thick and the lakes vary in salinity from completely freshwater (Lake Hoare) through weakly saline (Lake Fryxell) to hypersaline (Lake Bonney) (Spigel and Priscu 1998). The dominant bacterial nutrient cycling activities also differ among Taylor Valley lakes. For example, the water column of Lake Fryxell supports bacterial sulfur cycling (Sattley and Madigan 2006), while the water column of Lake Bonney lacks significant inorganic sulfur but instead supports vigorous nitrogen-cycling (Spigel and Priscu 1998; Ward and Priscu 1997).

In contrast to Taylor Valley lakes, Lake Vida, a large (6.8 km^2) lake in the Victoria Valley where the average temperature is about 10°C colder than in the Taylor Valley, is ice-sealed (that is, essentially free of liquid water), with an ice-cover 19 m thick (Doran et al. 2003). At a depth of about 16 m Lake Vida ice becomes a slush of Na, Mg and Ca salts with a temperature of −11.6°C; beneath this, the slush transits to a brine pocket at the bottom of the lake

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(Doran et al. 2003, 2008). Biogeochemical research on Lake Vida ice core samples indicates that both heterotrophic and phototrophic microbial processes occur (Doran et al. 2003, 2008), and molecular diversity studies have shown that several bacterial taxa are present (Mosier et al. 2006).

Although it is clear that Lake Vida ice contains bacteria, no isolates have yet been described. The present work addresses this issue through the isolation and characterization of five strains of heterotrophic bacteria from Lake Vida deep ice. The organisms obtained display physiological properties consistent with life in a constantly cold and salty environment (Deming 2009) and thus are likely derived from populations indigenous to this unusual polar lake.

Materials and methods

Sampling, enrichment, and isolation

In December of 2005 a small sample of Lake Vida 15.9 m ice was kindly supplied to MTM by Dr. Chris Fritsen, Desert Research Institute, Reno, NV (USA). The sample was maintained at 4°C in darkness for 5 months before enrichment cultures were established. For enrichments, 1-ml samples were added to 125-ml flasks containing 25 ml of medium R2A (Reasoner and Geldreich 1985) supplemented with 5 or 10% (w/v) NaCl and the flasks incubated aerobically without shaking at 4, 10 or 18°C. After 1 month, enrichments were streaked onto plates of the same medium and incubated at enrichment temperatures until isolated colonies appeared (ca. three weeks). In addition to plating primary liquid enrichments, dilutions of primary enrichments were made into 5 ml of 5% (w/v) or 10% NaCl-R2A medium in 17-ml screw-cap test tubes and the tubes incubated at the original enrichment temperatures for 1 month. Axenic cultures were subsequently obtained by plating.

Temperature and salt relationships

To determine the salt response of each strain, 3-ml aliquots of liquid medium R2A containing varying concentrations of NaCl were prepared in 10-ml screw-cap tubes. Triplicate tubes were inoculated and incubated at the enrichment temperature and growth was monitored turbidimetrically as OD_{660}. To determine the cardinal temperatures for growth, liquid media were prepared in 10-ml screw-cap tubes containing 3 ml of medium prepared at optimal salinity. Triplicate tubes were inoculated and incubated at −8 to +32°C (cultures did not freeze at subzero temperatures because of the salinity of the medium), and growth was monitored turbidimetrically as OD_{660}. Temperatures below ambient were obtained using Precision Scientific cooling boxes (Fisher Scientific, St. Louis, MO) with a thermometer placed near the culture tubes.

Molecular methods

Genomic DNA was extracted with a PureGene Genomic DNA Purification Kit (Gentra Systems, Minneapolis, MN). To amplify 16S rRNA genes, universal 16S rRNA bacterial PCR primers were used as previously described (Sattley and Madigan 2006). Prior to sequencing, all PCR products were purified using the GeneClean Turbo Kit (MP Biomedicals, Solon, OH). Automated DNA sequencing was performed at the Genome Sequencing Center, Washington University, St. Louis, MO. DNA sequence data were manually aligned with closely related 16S rRNA sequences obtained from a BLAST search using Se-Al v2.0 (http://tree.bio.ed.ac.uk/software/seal/). The phylogenetic tree was generated using the programs DNADIST (F84 algorithm: transition-transversion ratio 2.0, empirical base frequencies) and NEIGHBOR implemented in the PHYLIP version 3.68 (Felsenstein 1989). The program SEQBOOT was used in resampling for bootstrap analysis.

Results

Enrichment and isolation

Our major objective was to probe a sample of Lake Vida ice for aerobic heterotrophic bacteria using low temperatures and various salinities as enrichment variables, characterize the phylogeny of the isolates obtained, and assess their growth as a function of salt and temperature. Medium R2A was selected as a basal medium for all experiments because it contains relatively low levels of nutrients typical of icy environments (Deming 2009) and has been shown effective for the isolation of bacteria from nutrient-limited waters, including Antarctic melt-waters and subglacial ice (Christner et al. 2001; Reasoner and Geldreich 1985).

Liquid enrichment cultures established from Lake Vida ice at 4, 10, or 18°C became turbid within 1 month, and plating yielded isolated colonies in approximately three weeks. The 4°C plates contained three distinct colony types: large and small opaque colonies, and very small translucent colonies. These colonies yielded strains LV40, LV414 and LV411, respectively. Dilutions of primary liquid enrichments into fresh liquid medium and subsequent growth before plating yielded the other two Lake Vida isolates. Following growth of a 10^{-5} dilution of a 10% NaCl R2A (10°C) primary enrichment in the same medium, a single colony type was obtained and was the source of strain

Table 1 Summary of enrichment conditions and properties of Lake Vida isolates

Strain	Enrichment temp (°C)	Enrichment salinity (% NaCl)	Morphology/cell size (μm)/(motility)	Temp range (°C)	Temp optimum (°C)	Salinity range (% NaCl)	Salinity optimum (% NaCl)	Cold/salt phenotype
Psychrobacter relatives[a]								
LV181	18	10	Rod, 0.6 × 1.0 (+)	−4 to +25	8	2–13	10	Psychrotolerant/ halophilic
LV40	4	5	Coccus, 0.8–0.9 (−)	−8 to +18	0–8[b]	0–10	5	Psychrophilic/ halotolerant
LV414	4	5	Coccus, 0.5–0.6 (−)	−4 to +15	8	2–10	5–10[b]	Psychrophilic/ halophilic
Marinobacter relatives[a]								
LV10S	10	10	Rod, 0.8 × 1.1 (+)	−4 to +25	14	2–15	10	Psychrotolerant/ halophilic
LV411	4	5	Rod, 0.3 × 1.1 (+)	−4 to +20	4–8[b]	2–15	2–5[b]	Psychrophilic/ halophilic

Medium R2A and aerobic incubations used in all cases. Temperature experiments done at optimal salinity for each strain

[a] See Fig. 1

[b] Mean growth yields in triplicate cultures incubated for a defined time period were virtually the same at all temperatures or salinities shown as a range (Fig. 2 inset)

LV10S. A 10^{-5} dilution of the 10% NaCl R2A (18°C) enrichment and subsequent growth and plating yielded shiny transparent colonies that were the source of strain LV181. A summary of enrichment conditions and basic properties of the Lake Vida ice isolates is given in Table 1.

Morphology and phylogeny of the Lake Vida ice isolates

Microscopic observation of cells of each isolate revealed them to be either rods or cocci; the rods were motile, while the cocci were not (Table 1). The Gram reaction as assessed by both staining and by KOH treatment showed all strains to be gram-negative bacteria.

Based on SSU rRNA gene sequences, all Lake Vida isolates were species of *Gammaproteobacteria*, either of the genera *Psychrobacter* or *Marinobacter* (Fig. 1). The closest relatives of strain LV181 were three *Psychrobacter* species: *Psychrobacter luti*, isolated from the South Shetland Islands in the sub-Antarctic zone (Bozal et al. 2003), *Psychrobacter glacincola*, a halotolerant, psychrophilic bacterium from Antarctic sea ice (Bowman et al. 1997a, b), and *Psychrobacter okhotkensis*, a psychrotolerant bacterium isolated from the Okhotsk Sea in Japan (Yumoto et al. 2003). The closest relative to Lake Vida strains LV40 and LV414 was the species *Psychrobacter namhaensis*, a halotolerant cold-active organism from the Korean South Sea (Yoon et al. 2005). By contrast, strains LV10S and LV411 were *Marinobacter* species and clustered together tightly on the tree (Fig. 1). The closest cultured relative of these Lake Vida isolates was *Marinobacter maritimus*, a psychrotolerant bacterium isolated from subantarctic seawater (Shivaji et al. 2005).

Temperature and salt relationships of the Lake Vida isolates

To determine whether the Lake Vida strains were psychrotolerant (growing optimally above 20°C but capable of growth at 0°C) or truly psychrophilic (growing optimally at 15°C or lower and incapable of growth above 20°C) (Cowan et al. 2007; Deming 2009), growth experiments at various temperatures were conducted. The results showed that examples of both phenotypes had emerged from the enrichments. Three of the five Lake Vida ice isolates were psychrophilic *sensu stricto*, while two were psychrotolerant, but all five strains showed growth temperature optima below 20°C and could grow at temperatures below 0°C (Table 1). Although the psychrotolerant strains grew at 25°C, they did not grow at temperatures above this. All psychrophilic isolates were obtained from enrichments established at 4°C, while both psychrotolerant strains emerged from higher enrichment temperatures (Table 1).

Strain LV40 was the most cold active of all isolates, growing slowly at −8°C and incapable of growth above 18°C (Table 1; Fig. 2). The doubling time of strain LV40 at 4°C was about 36 h, while that at −4°C was about 8 days; at −8°C the doubling time of this strain was approximately 10 days (Fig. 2). Strain LV40 also showed the lowest growth temperature optima of all strains isolated, with quite similar growth rates occurring at temperatures from 0 to 8°C (Table 1; Fig. 2 inset).

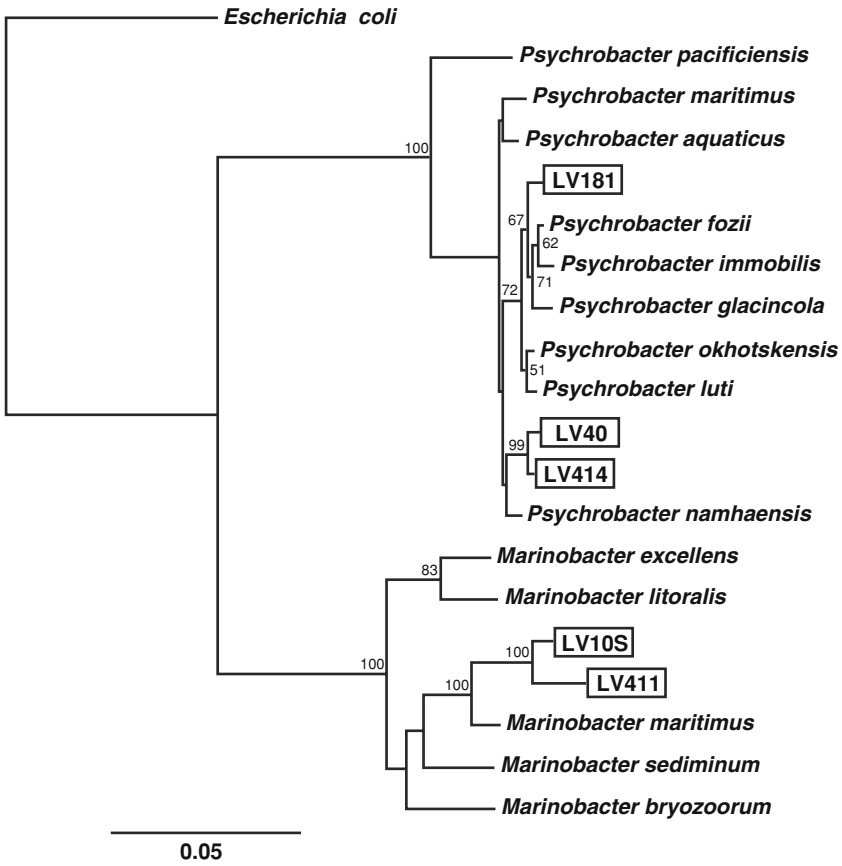

Fig. 1 Phylogeny of Lake Vida isolates based on comparative SSU ribosomal RNA sequences. The tree was generated from alignments of 1,077 nucleotides. *Numbers* at nodes indicate bootstrap values >50% from 1,000 replications. Further properties of each isolate are given in Table 1

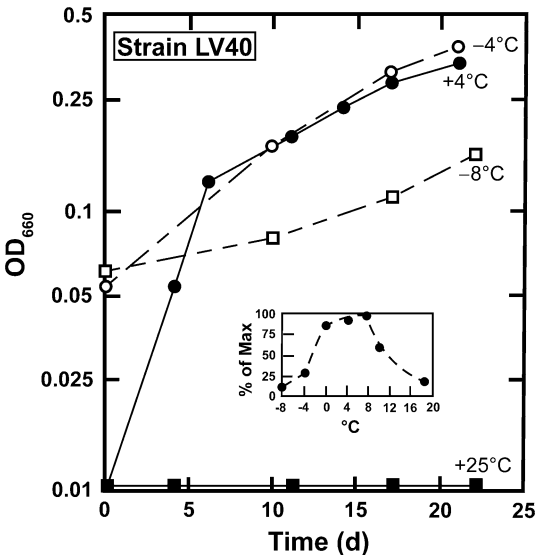

Fig. 2 Growth of Lake Vida strain LV40 at −8, −4, and +4°C in medium R2A containing 5% NaCl. Turbidity values (OD_{660}) are the mean of triplicate cultures at each temperature. *Inset* Growth of strain LV40 as a function of temperature. Cultures were grown for 17 days at the temperatures indicated and the cell yields compared. Values are the average of triplicate cultures at each temperature

Salinity requirements and tolerances varied among the Lake Vida isolates. Except for strain LV40, all strains were halophilic; that is, they had an absolute salt requirement for growth (Table 1). The upper salinity limit for growth of the halophilic Lake Vida strains was between 10 and 15% NaCl, and all strains showed salinity optima for growth between 5 and 10% NaCl (Table 1). Notably, even strain LV40, which could be transferred repeatedly in media lacking salt and was therefore halotolerant instead of halophilic, still grew optimally in hypersaline media (Table 1).

Discussion

Our work is the first to describe pure cultures of bacteria from Lake Vida ice. Our major conclusions are that all isolates grew best under cold and saline conditions, consistent with the properties of their habitat (Doran et al. 2003, 2008), and that their phylogeny is predictive of a cold-active and halotolerant phenotype.

Our experiments could not reproduce in situ temperature conditions of Lake Vida ice precisely, as we were unable to reliably obtain temperatures below −8°C. But it is distinctly possible that our strain LV40 can grow below −8°C, and if so, this would approach the in situ temperature of Lake Vida brine ice. Supporting this contention is the fact that a Siberian permafrost *Psychrobacter* species, *P. cryohalolentis*, related to *P. glacincola* grew at −10°C

(Bakermans et al. 2006) and an Alaskan sea-ice *Psychromonas* species, *P ingrahamii*, at −12°C (Auman et al. 2006). Strain LV40 is closely related to *P. glacincola* (Fig. 1) and *Psychrobacter* and *Psychromonas* are fairly closely related *Gammaproteobacteria*. In addition, an uncharacterized *Psychrobacter* species has been shown to metabolize down to at least −15°C (Christner 2002) Thus, it is clear that at least some representatives of the *Psychrobacter*/*Psychromonas* cluster can grow at the prevailing temperatures of Lake Vida ice, and strain LV40 may well be a new example in this regard.

Salinity tolerance of our isolates is another issue, however. In pure cultures, the upper salinity limit for growth of the Lake Vida strains ranged from 10 to 15% NaCl. Even the latter value is significantly lower than the salinity of the Lake Vida ice from which the isolates were enriched (>20% total salinity, Doran et al. 2003). However, it should be noted that in addition to NaCl as major salt, the briny Lake Vida ice contains significant levels of Mg^{2+} K^{+}, and SO_4^{2-} (Doran et al. 2003). Thus, it is possible that specific salt mixtures could be found that support growth of Lake Vida bacteria at total salinities higher than those that support growth using NaCl alone.

All of the Lake Vida isolates were relatives of the genera *Psychrobacter* or *Marinobacter*; these bacteria are common in marine and polar-regions (Bowman et al. 1997a, b; Bozal et al. 2003; Tindall 2004; Yoon et al. 2005; Zhang et al. 2008). Classic characteristics of species of these genera include cold and salt tolerance (Bowman 2008; Gauthier et al. 1992; Yoon et al. 2003; Zhang et al. 2008), a combination of phenotypes that should be ideal for life in the briny Lake Vida ice. Phylotypes related to several *Marinobacter* species and other *Gammaproteobacteria* were detected in molecular diversity studies of an ice core from Lake Vida, in particular from the deeper ice such as was used for enrichment purposes here (Mosier et al. 2006). Curiously, however, no phylotypes closely related to the *Psychrobacter* cluster that included our strains LV181, LV 40, and LV414 (Fig. 1) emerged from these community-sampling experiments (Mosier et al. 2006).

From the foregoing, we predict that the Lake Vida isolates obtained in this study are derived from indigenous bacterial populations that reproduce in this unusual polar lake rather than simply being transients blown in from the nearby hills. Assuming this is true, in situ growth rates of these organisms are likely to be extremely low, not only because of the low temperature and high salinity of Lake Vida ice, but also because only traces of dissolved organic carbon are present in the ice (Doran et al. 2003). Thus, doubling times for bacteria inhabiting Lake Vida ice could be on the order of months, or even years. Our isolates serve as a starting point for the study of bacterial life in ice-sealed lakes and their combination of salt and cold tolerance helps further stretch our definition of the physiochemical limits to life on Earth, and possibly on other, even colder planets.

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References

Auman AJ, Breezee JL, Gosink JJ, Kämpfer P, Staley JT (2006) *Psychromonas ingrahamii* sp. nov., a novel gas vacuolate, psychrophilic bacterium isolated from Arctic polar sea ice. Int J Syst Evol Microbiol 56:1001–1007

Bakermans C, Ayala-del-Río HL, Ponder MA, Vishnivetskayr T, Gilichinsky D, Thomashow MF, Tiedje JM (2006) *Psychrobacter cyrohalolentis* sp. nov. and *Psychrobacter arcticus* sp. nov., isolated from Siberian permafrost. Int J Syst Evol Microbiol 56:1285–1291

Bowman JP (2008) The genus *Psychrobacter*. In: Dworkin M et al (eds) The prokaryotes: an evolving electronic resource for the microbiological community, 3rd edn. Springer, New York, http://link/springer-ny.com/link/service/books/10125/

Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA (1997a) Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl Environ Microbiol 69:3068–3078

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

Bozal N, Montes MJ, Tuleda E, Guinea J (2003) Characterization of several *Psychrobacter* strains isolated from Antarctic environments and description of *Psychrobacter luti* sp. nov. and *Psychrobacter fozii* sp. nov. Int J Syst Evol Microbiol 53:1093–1100

Christner BC (2002) Incorporation of DNA and protein precursors into macromolecules by bacteria at −15°C. Appl Environ Microbiol 68:6435–6438

Christner BC, Mosley-Thompson E, Thompson LG, Reeve JN (2001) Isolation of bacteria and 16SrDNAs from Lake Vostok accretion ice. Environ Microbiol 3:570–577

Cowan DA, Casanueva A, Stafford W (2007) Ecology and biodiversity of cold-adapted microorganisms. In: Gerday C, Glansdorff N (eds) Physiology and biochemistry of extremophiles. American Society for Microbiology Press, Washington, DC, pp 119–132

Deming J (2009) Extremophiles: cold environments. In: Schaechter M (ed) Encyclopedia of microbiology, vol VI. Elsevier, Oxford, pp 147–158

Doran PT, Fritsen CH, McKay CP, Priscu JC, Adams EE (2003) Formation and character of an ancient 19-m ice cover and underlying trapped brine in an "ice-sealed" east Antarctic lake. Proc Natl Acad Sci (USA) 100:26–31

Doran PT, Fritsen CH, Murray AE, Kenig F, McKay C, Kyne JD (2008) Entry approach into pristine ice-sealed lakes—Lake Vida, East Antarctica, a model ecosystem. Limnol Oceanog Methods 6:542–547

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

Fountain AG, Lyons WB, Burkins MB, Dana GL, Doran PT, Lewis KJ, McKnight DM, Moorhead D, Parsons AN, Priscu JC, Wall

DH, Wharton RW, Virginia RA (1999) Physical controls on the Taylor Valley ecosystem, Antarctica. Bioscience 49:961–971

Gauthier MJ, LaFay B, Christen R, Bonin P, Bertrand J-C (1992) *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. Int J Syst Bacteriol 42:568–576

Gordon DA, Priscu JC, Giovannoni S (2000) Origin and phylogeny of microbes living in permanent Antarctic lake ice. Microb Ecol 39:197–202

Matsumoto GI (1993) Geochemical features of the McMurdo Dry Valley lakes, Antarctica. Ant Res Series 59:95–118

Mosier AC, Murray AE, Fritsen CH (2006) Microbiota within the perennial ice cover of Lake Vida, Antarctica. FEMS Microbiol Ecol 59:274–288

Priscu JC (ed) (1998) Ecosystem dynamics in a polar desert: the McMurdo Dry Valleys, Antarctica. Ant Res Series Vol 72. American Geophysical Union, Washington, DC

Priscu JC, Fritsen CH, Adams EE, Giovannoni SJ, Paerl HW, McKay CP, Doran PT, Gordon DA, Lanoil BD, Pinckney JL (1998) Perennial Antarctic lake ice: an oasis for life in a polar desert. Science 280:2095–2098

Priscu JC, Wolf CF, Takacs CD, Fritsen CH, Laybourn-Parry J, Roberts EC, Lyons WB (1999) Carbon transformations in the water column of a perennially ice-covered Antarctic Lake. Bioscience 49:997–1008

Reasoner DJ, Geldreich EE (1985) A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol 49:1–7

Sattley WM, Madigan MT (2006) Isolation, characterization, and ecology of cold-active, chemolithotrophic, sulfur-oxidizing bacteria from perennially ice-covered Lake Fryxell, Antarctica. Appl Environ Microbiol 72:5562–5568

Shivaji S, Gupta P, Chaturvedi P, Suresh K, Delille D (2005) *Marinobacter maritimus* sp. nov., a psychrotolerant strain isolated from sea water off the subantarctic Kerguelen islands. Int J Syst Evol Microbiol 55:1453–1456

Spigel RH, Priscu JC (1998) Physical limnology of McMurdo Dry Valley lakes. In: Priscu JC (ed) Ecosystem dynamics in a polar desert, vol 72. American Geophysical Union, Washington, DC, pp 153–187

Tindall BJ (2004) Prokaryotic diversity in the Antarctic: the tip of the iceberg. Microb Ecol 47:271–283

Ward BB, Priscu JC (1997) Detection and characterization of denitrifying bacteria from a permanently ice-covered Antarctic lake. Hydrobiology 347:57–68

Yoon JH, Shin DY, Kim IG, Kang KH, Park YH (2003) *Marinobacter litoralis* sp. nov., a moderately halophilic bacterium from sea water from the East Sea in Korea. Int J Syst Evol Microbiol 53:563–568

Yoon JH, Lee CH, Yeo SH, Oh TK (2005) *Psychrobacter aquimaris* sp. nov. and *Psychrobacter namhaensis* sp. nov., isolated from sea water of the South Sea in Korea. Int J Syst Evol Microbiol 55:1007–1013

Yumoto I, Hirota K, Sogabe Y, Nodasaka Y, Yokota Y, Hoshino T (2003) *Psychrobacter okhotkensis* sp. nov., a lipase-producing facultative psychrophile isolated from the coast of the Okhotsk Sea. Int J Syst Evol Microbiol 53:1985–1989

Zhang DC, Li HR, Xin YH, Chi ZM, Zhou PJ, Yu Y (2008) *Marinobacter psychrophilus* sp. nov., a psychrophilic bacterium isolated from the Arctic. Int J Syst Evol Microbiol 58:1463–1470