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Detection and characterization of denitrifying bacteria from a permanently ice-covered Antarctic Lake

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Abstract

Denitrifying bacterial strains were isolated from Lake Bonney, a permanently ice-covered and chemically stratified lake in the McMurdo dry valley region of Antarctica, using complex media at 4 °C. Three strains, identified as denitrifiers by their ability to produce nitrous oxide using nitrate or nitrite as a respiratory substrate, were characterized as to their temperature and salinity optima for aerobic growth in batch culture; all three were psychrophilic and moderately halophilic. Maximum growth rates of near 0.024 h^{-1} were measured for all three strains. Growth rates projected to occur at *in situ* temperature and salinity imply generation times on the order of 100 h. Species specific polyclonal antisera were prepared against two of the strains, ELB 17 (from the east lobe of the lake at 17 m) and WLB20 (from the west lobe at 20 m). Both strains were subsequently detected and enumerated in the lake using the antisera. ELB17 was present in both lobes below the chemocline, while WLB20 was present in the west lobe below the chemocline but only in surface waters of the east lobe. These distributions are related to the observed chemical distributions which imply the occurrence of denitrification in the west lobe of the lake and not in the east lobe.

Introduction

Although the physiological characteristics of nitrifying and denitrifying bacteria are apparently incompatible, there are many environments in which coupled nitrification/denitrification is the rule. These environments are characterized by physicochemical gradients across which the substrates and products of the coupled reactions diffuse and which allow the incompatible transformations to be separated in time or space. Examples of such gradient systems, and the typical chemical distributions which result, are the Black Sea (Codispoti et al., 1991) and aquatic sediment environments (Reeburgh, 1983). In the water column of lakes and oceans, the transition from oxygenated water to anoxic water occurs over meters to hundreds of meters, while in sediments, the relevant scale is microns to centimeters. In what may be considered a 'normal' situation, the upper layer contains nitrate (and often oxygen); below the interface, nitrate and oxygen are both totally depleted and ammonium and sulfide may accumulate as the result of anaerobic decomposition. Small peaks in nitrite and nitrous oxide are often present near the interface. These chemical distributions are interpreted as the result of stratification in the activities of nitrifying (aerobic), denitrifying (facultatively anaerobic) and sulfate reducing (usually anaerobic) bacteria. The lake where this study was performed has two lobes, both stably stratified and connected by a shallow sill. The west lobe exhibits the classical chemical distributions described here, whereas the east lobe exhibits anomalous nitrogen distributions, specifically, high concentrations of oxidized nitrogen in suboxic water (Priscu et al., 1996).

Permanently ice-covered Lake Bonney, an Antarctic lake in the Taylor Valley adjacent to McMurdo

Sound, presents a special situation where turbulence and upper trophic levels are virtually nonexistent. The lake is chemically stratified; the chemocline divides the lake into an upper trophogenic zone and a lower zone where photoautotrophic activity is virtually absent (Lizotte et al., 1996; Priscu, 1995). Thus, physical processes (mainly diffusion) and microbial activity dominate biogeochemica] reactions and chemical distributions, particularly below the chemocline, which coincides with the oxycline. The deep water chemistry of the two lobes of the lake differs dramatically (Spigel & Priscu, 1996), probably due ultimately to important differences in the recent (1000–2000 yr) histories of the two lobes (Lyons et al., in press). The lobes are separated by a sill depth of 12–13 m and both lobes are perennially covered by 3–5 m of ice. Phytoplankton primary productivity in both lobes is restricted to the layer above the chemocline; 20 m in the east lobe and 15 m in the west lobe (Priscu, 1995; Lizotte et al., 1996). Productivity maxima are associated with nutrient gradients within this layer. In the west lobe, the distribution of inorganic nitrogen species below the chemocline is consistent with a typical stratified system in which the surface layer is nitrogen-depleted, and the deep anoxic layer has high ammonium concentrations, but nitrate present at very low concentrations, apparently depleted by denitrification. Denitrification has been detected in water from the west lobe by the acetylene block method (Priscu et al., 1996). The east lobe also has a nitrogen-depleted surface layer and an anoxic deep layer. This deep layer, in contrast to the west lobe, has high concentrations of nitrate, nitrite and ammonium, making it appear that denitrification has not occurred at rates high enough to deplete the oxidized nitrogen pool. Denitrification was not detectable in the east lobe by the acetylene block method (Priscu et al., 1996). At the oxic/anoxic interface of the east lobe, nitrous oxide occurs at very high levels, but much lower levels exist in the west lobe (Priscu et al., 1993; Priscu et al., 1996).

These observations suggest that denitrification is occurring below the chemocline in the west lobe but not in the east and that nitrification, rather than denitrification, dominates the chemical distributions in the east lobe. This difference could be due simply to absence of denitrifying bacteria in the east lobe, or to inhibition of denitrification in the east lobe. Even if the chemical distributions are historical and do not reflect modern processes (Priscu, 1995), the exact reason for lack of denitrification in the east lobe remains in question. A first step in discerning the underlying mechanisms is to determine the distribution of bacteria responsible for the processes of nitrification and denitrification, which are thought partially to control the nutrient distributions. Denitrifying bacteria are addressed specifically in this paper. Measurements of rate transformations (Priscu et al., 1996) and nitrifying bacterial distributions (Voytek & Ward, 1995; Voytek, 1996; Voytek, Ward & Priscu, in preparation) are presented elsewhere.

Methods

Isolations and strains

Two approaches were used for isolation of denitrifying strains from lakewater. Strain ELB17 was isolated from 17 m in the east lobe by adding a complex carbon source (peptone, 1 g l^{-1}) and nitrate (1 mM KNO_3) to natura] lake water, which was then incubated at *in situ* temperature or at 4 °C. Strains WLB20 (20 m in the west lobe) and WLB35 (35 m in the west lobe) were isolated by inoculating 1 ml of lake water into 25 ml of sterile artificial lake water medium, which had been enriched with peptone and nitrate as above. The artificial lake water medium was made from distilled water plus 1 ml trace metals solution (Biebl & Pfennig, 1978) plus the following salts (g l^{-1}) in concentrations designed to cover the range of salinities found in the lake (Spigel & Priscu, 1996): $MgSO_4.7H_20O$: 46.3 and 65.9; NaCl: 62.5 and 85; $CaSO_4.2H_2O$: 4.65 and 5.12, for WLB20 and WLB35, respectively. Isolates were streaked to purity on agar plates of the same medium under an atmosphere of dinitrogen gas. In order to encourage the growth of strains adapted to the environment of origin, enrichments were maintained as close as possible to the original ambient temperature (4 °C) until pure isolates were obtained. The isolates were then frozen in 15% glycerol at −70 °C until required for experiments. Subsequent growth of the isolates for production of antigens took place at a higher temperature in order to obtain more rapid growth (12 °C).

Ability of isolates to denitrify was ascertained by sequential growth in the above medium on oxygen, then nitrate, then nitrite as electron acceptors. Ability to grow on NO_2 (0.01 to 0.25% w/v) and detection of trace levels of N_2O (by ECD gas chromatography) in the headspace of sealed tubes in which liquid cultures were grown were taken as evidence of ability to denitrify completely to nitrogen gas.

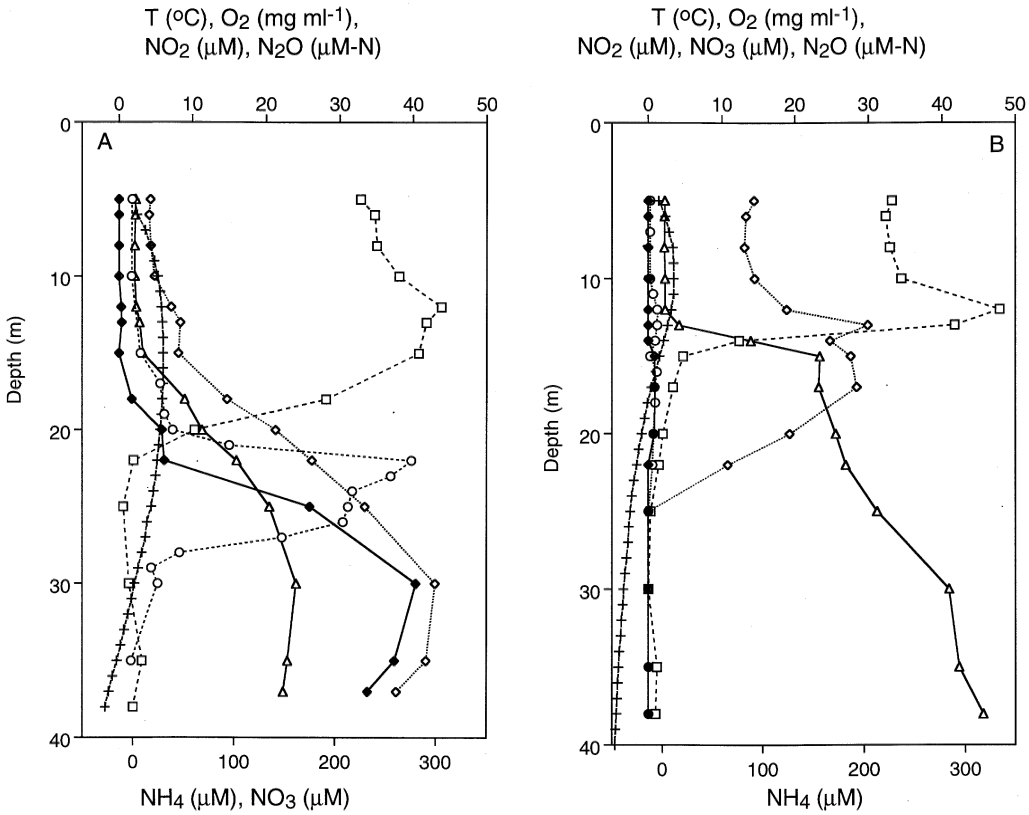

Figure 1. Hydrographic data (November 1994) from central stations in (A) East and (B) West lobes of Lake Bonney. Temperature (+); oxygen (□); nitrite (◇); ammonium (△); nitrate (◇); nitrous oxide (○).

Growth rate experiments

Inocula for the growth rate experiments were grown in medium of ambient salt concentration at 12 °C until turbidity was detected. One ml of log phase culture was then inoculated into each of 16 treatment flasks, each containing 100 ml of artificial lake water medium of identical composition except for salt concentration. The treatments consisted of 4 different NaCl concentrations representing approximately 0.25×, 0.5×, 1× and 2× relative to ambient (1×). (Ambient salt [NaCl + $MgSO_4$ + $CaSO_4$] levels for the three isolates were 60, 99, and 136 g l^{-1} for ELB17, WLB20 and WLB35 respectively.) One flask of each of the different salt concentrations was incubated at one of four different temperatures: 4 °C, 12 °C, 15 °C, 25 °C. Flasks were shaken continuously at about 125 rpm. At least once daily, a 1 ml sample was removed aseptically from each flask and its OD measured in a small volume cell at 450 nm using a Hitachi dual beam spectrophotometer. For each flask, a log transformation of the OD data was made and a maximum growth rate was determined from the slope of the regression equation from the linear portion of the log plot during early exponential phase.

Immunofluorescence and bacterial enumeration

For production of antibodies, cells were grown at 12 °C aerobically in 1-L flasks in medium of ambient salt concentration and harvested by centrifugation. Cell pellets were washed twice with phosphate buffered saline (PBS; per liter of distilled water: 8 g NaCl, 0.2 g KCl, 1.15 g Na_2HPO_4, 0.2 g KH_2PO_4), resuspended in a minimum volume of PBS and frozen. The protein concentration of the antigen preparation was adjusted to 10 mg ml^{-1} (by dilution with PBS) as measured by the Coomassie blue assay (Bradford, 1976).

Antibodies for ELB17 were produced in rabbits by the Berkeley Antibody Company (Berkeley, CA) and for WLB20 by Rockland, Inc. (Boyertown, PA) following the immunization protocol of Ward and Cockcroft

(1993). The working antibody concentration (800-fold dilution for ELB17, 400-fold dilution for WLB20) was chosen as the highest dilution which resulted in a + 4 reaction by immunofluorescence (on an operator subjective scale from undetectable (−) to maximum fluorescence intensity (+ 4)) and which resulted in + 4 staining of every cell in a sample from a homologous culture.

Specificity of the antibodies was investigated by immunofluorescence staining of a variety of known denitrifying (15 strains) and non-denitrifying strains (11 strains), and a group of recently isolated unidentified denitrifiers (9 strains), using the microfuge staining method described previously (Ward & Carlucci, 1985). The strains we tested are those used by Ward and Cockcroft (1993) to test the specificity of another denitrifier antiserum, plus two additional environmental isolates. The antisera were found to be very specific (no cross reactions with heterologous strains). To minimize nonspecific staining in complex samples, both antisera were absorbed with whole cells of E. colz before using them to enumerate natural samples (Belly et al., 1973). Absorption of the antiserum did not reduce the number or intensity of stained cells in the pure culture of target cells. Field samples were stained only with absorbed serum.

Indirect immunofluorescence (IIF) staining methods for water samples have been described previously (Ward & Carlucci, 1985). The fluorescent antibody, fluorescein isothiocyanate-conjugated sheep anti-rabbit immunoglobulin G, was obtained from Miles Laboratories, Inc. (Elkhardt, IN). The microfuge method was used for cross reaction tests and the filter staining method (using 0.2 μm pore size, 25 mm Nuclepore or Poretics filters, pre-stained in Irgalan Black) was used for enumeration of formalin preserved (see below) lake samples (Ward & Carlucci, 1985). Stained samples were stored in the refrigerator and retained their initial fluorescence almost indefinitely. Samples were viewed by epifluorescence microscopy at × 1000 power using a Zeiss standard microscope with epifluorescence modification (50 W Hg illumination; 450 DF 55 filter for excitation, 505 DF 35 barrier filter and 505DRLEXT02 dichroic filter). Immunofluorescently stained cells were distinguished in natural samples by a staining reaction of + 4 or + 3 (compared to the homologous reaction of + 4). ELB17 cells have a characteristic long thin rod shape and WLB20 cells were quite distinct in shape, being much shorter and thicker. It was possible to enumerate both kinds of cells on the same filter by staining with a mixture of the two sera, but it was simpler to enumerate them separately. For enumeration of water samples, 10–20 ml samples were counted. For both AODC (see below) and IIF staining, a total of 300 fields or approximately 200 cells were enumerated for each count. This did not always result in enumeration of a sufficient number of cells to exceed the limit of detection (in the case of IIF, it was not always possible to count 200 cells), but represents a maximum acceptable amount of time and effort per sample. Acridine orange stained filters were prepared from 5–10 ml aliquots of the same samples and enumerated using the same microscope with the same filter set (AODC, Porter & Fieg, 1980).

Ancillary chemical measurements and sources of materials

Samples for enumeration of bacteria from planktonic communities were collected in November and December of 1992, 1993 and 1994 using Niskin sampling bottles lowered through holes in the 4-m thick layer of ice covering the surface of the lake. Approximately 200 ml was collected in previously combusted glass bottles to which 4 ml filtered (0.22 μm) buffered (sodium borate) formalin were added. Bottles were stored in the dark at 4 °C until enumeration.

Oxygen concentrations were determined by Winkler titrations (Parsons et al., 1984). Dissolved nutrients (nitrate, nitrite and ammonium) were assayed by standard methods (Parsons et al., 1984) with slight modifications to allow for minimal sample sizes. Conductivity and temperature were measured with a Martek 7 instrument (Spigel & Priscu, 1996). N_2O samples were collected directly from a 5-liter Niskin bottle connected to a 10 ml glass-barrel syringe with a 2 cm length of rubber tubing. Every precaution was taken to ensure that the samples did not contact air or otherwise degas. Water samples (5 ml) were analyzed following 2 or 3 equilibrations with high-purity He (McAuliffe, 1971). N_2O was measured with a Perkin-Elmer Sigma 4 gas chromatograph fitted with a 2 m stainless steel column of Chromosorb 102 (100–120 mesh) and operated at a column temperature of 56 °C, with a carrier gas (95% Ar: 5% CH4) flow of 24 ml min^{-1}. The instrument was standardized as outlined by Priscu et al. (1996).

During isolation and culturing, nitrite was assayed by the spectrophotometric method of Parsons et al. (1984) using a Hitachi double beam spectrophotometer. Nitrous oxide was detected using a Shimadzu Mini-2 gas chromatograph equipped with an electron capture

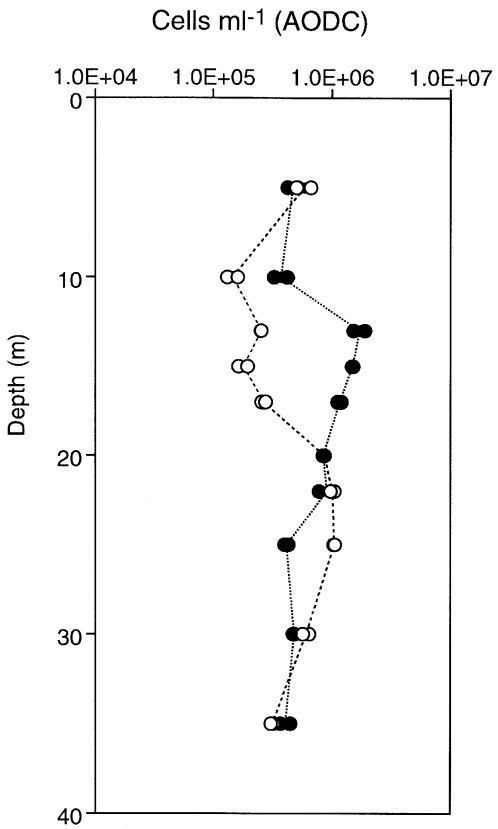

Figure 2. Total bacterial abundance in East (○) and West (●) lobes of Lake Bonney. Means (+) and replicate counts (○, ●) from November 1994.

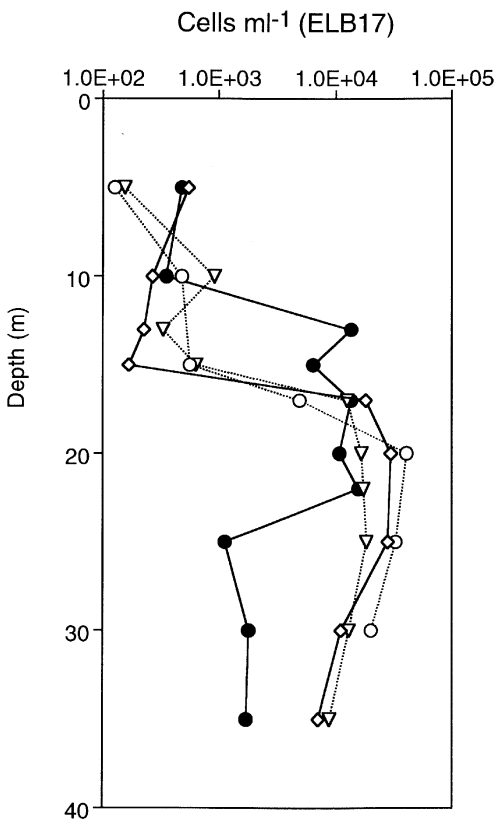

Figure 3. Abundance of ELB 17 from IIF assay. Data from November 1994 (counts from three casts for the east lobe samples). East lobe (○, ◇, △); West lobe (●).

detector and a 2 m Poropak Q column run at an injection temperature of 300 °C and a column temperature of 45 °C. Gas standards were obtained from Scott Specialty Gases (San Bernardino, CA).

Results

Hydrographic distributions

Lake Bonney consists of two elongate lobes, each with maximum depth of approximately 40 m, separated by a sill of 13 m depth. All data presented in this paper were collected from the central stations in each lobe, designated E30 (East lobe) and W20 (West lobe). Although there is slight variability among years, the nutrient and chemical data show consistent permanent stratification (Figure 1). Depths here refer to depth below the hydrostatic water level (i.e. the water level inside the sampling hole). The halocline and oxycline co-occur in the region of 13–15 m in the west lobe and at 17–20 m in the east lobe. Nutrient and hydrographic distributions have not varied significantly below the chemocline in at least ten years (Spigel & Priscu, 1996). The water level of the lakes has risen perceptibly in that time, but this has resulted from addition of freshwater to the upper layer and has not significantly affected the distribution of variables discussed here. As previously noted (Priscu et al., 1993), the two lobes differ strikingly in the concentrations of inorganic nitrogen species in the deep waters. Nitrate and nitrite are absent from subhalocline waters in the west lobe, while both are present at very high levels at similar depths in the east lobe. Nitrous oxide concentrations also differed between the two lobes, being present at very low levels in the west lobe and at concentrations exceeding 40 μM N_2O-N (Priscu et al., 1996) in the interface region of the east lobe. Maximum temperatures of 5.9 °C and 2.9 °C were observed at 18 and 10 m in the east and west lobes, respectively.

Distribution of total bacteria and denitrifying strains

Depth profiles of bacterial abundance (AODC) in both lobes of Lake Bonney in 1994, the year from which the most complete data set was obtained, are shown in Figure 2. Data are from a single sample (duplicate subsamples counted) for each depth from a profile collected in November 1994. In the east lobe, highest cell numbers (1×10^6 ml^{-1}) were found below the chemocline and in the west lobe, maximum numbers (nearly 2×10^6 ml^{-1}) occurred in the chemocline near 15 m. In the east lobe, the bacterial abundance maximum was broad and extended from about 20 to 30 m. In the west lobe, the upper boundary of the abundance maximum was sharply defined near the chemocline and numbers exceeded 1×10^6 ml^{-1} between 13 and 20 m.

The distribution of ELB17, the denitrifier originally isolated from 17 m in the east lobe, is shown in Figure 3. For the immunofluorescence counts, each point represents a single count (replicate counts on the same sample were not made due to the large amount of time required for staining and counting of 350 fields); each profile represents samples from a different date over the period of 4 weeks in November and December 1994. Very few ELB17 cells were detected in the upper layer but a broad maximum occurred below the chemocline. This maximum just above the chemocline coincided roughly with the maximum in total cell numbers but highest abundances of ELB17 were in the range of 2.5–4 $\times 10^4$ cells ml^{-1}. ELB17 was also detected in the west lobe with a broad maximum between 13 and 20 m, corresponding to the shallower chemocline in this lobe.

WLB20 was detected in both lobes but with very different distributions (Figure 4). In the west lobe, WLB20 had a maximum of only 1×10^3 cells ml^{-1} near 15 m, just below the chemocline, and was present throughout the deep layer at the level of a few hundred cells per ml. In the east lobe, WLB20 was present mainly above the chemocline forming a discrete maximum of up to 1×10^4 cells ml^{-1} at 17 m, but present at generally less than 100 cells ml^{-1} at both shallower and deeper depths.

Optimal growth conditions for Lake Bonney denitrifying isolates

The three isolates all exhibited classical growth curves, with variable lag times and maximal growth rates, the magnitude of which depended on temperature and salinity. An example of the range of growth curves obtained in the 16 salinity/temperature combinations is shown for strain WL20 in Figure 5. Although the isolates were never exposed to temperatures greater than 4 °C during the isolation procedure, and the temperature of the lake environment from which they were isolated never exceeded 6 °C, maximum growth rates occurred between 12 and 15 °C in all three isolates (Table 1). The optimum temperature (the temperature which allows maximum growth rate) appears to be in this range because lower growth rates were observed at both higher and lower temperatures, but the resolution of temperatures tested did not allow the identification of the single best temperature. Isolates were never grown in medium containing a salt concentration different from their environment during the entire isolation procedure. The absolute salt concentration at which maximal growth rates occurred differed for the three isolates (Table 1), but in each case was below the salt concentration of the water from which they were isolated. Contour plots derived from the observed growth rates for each isolate at each temperature and salinity combination (Figure 6) suggest that both WLB20 and WLB35 might have optimal salt concentrations below those tested in the matrix of culture conditions. It was not determined whether the isolates had an absolute salt requirement; the focus of the experiments was to relate growth of the organisms to *in situ* conditions, which included relatively high salt concentrations.

Maximum growth rates obtained under optimal conditions were about 0.024 h^{-1} for all three isolates, which translates into generation times of around 40 h. Growth rates estimated to occur at ambient conditions could be estimated from the contour plots of growth rate as a function of temperature and salinity (Figure 6). Rates estimated for in situ temperature and salinity ranged from 0.011 h^{-1} for ELB17 to no growth for WLB35. These values cannot be estimated precisely because growth experiments were not performed at temperatures below zero. (For the same reason, the expected growth rates for WLB20 and WLB35 under ambient conditions cannot be shown on the contour plots (Figure 6)). The data indicate, however, that, growth rates at *in situ* conditions are most likely to be much slower than those at optimal conditions.

Discussion

Both denitrifying isolates for which antisera were produced were subsequently detected in the lake, verify-

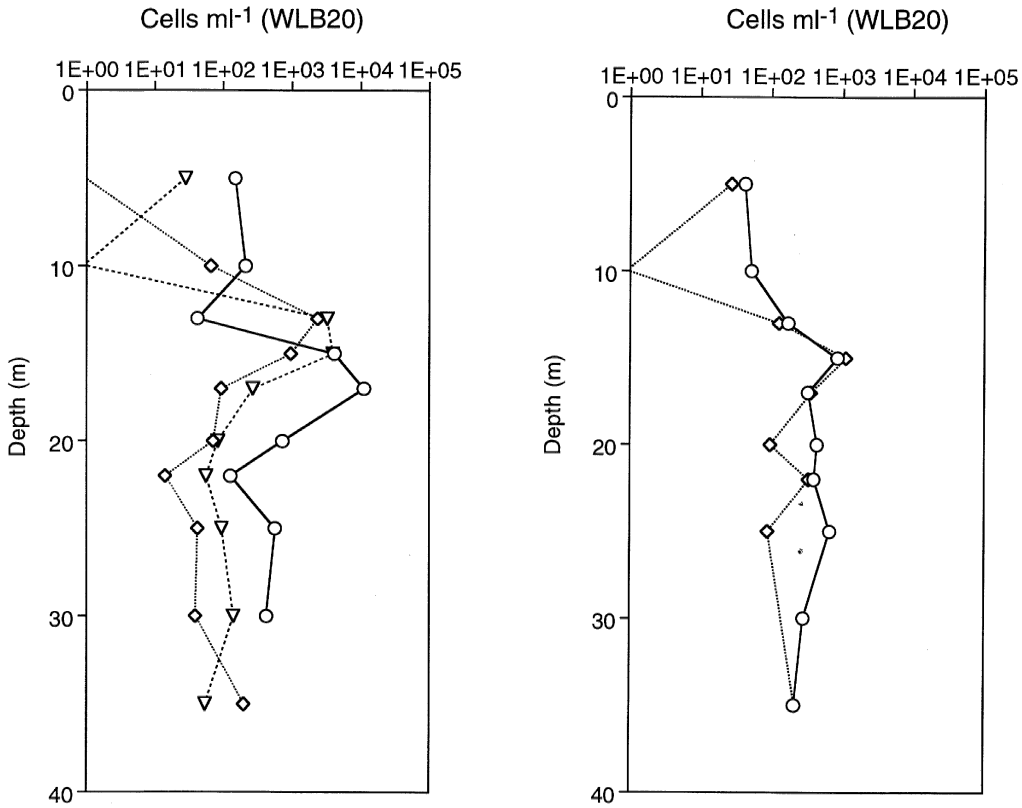

Figure 4. Abundance of WLB20 from IIF assay. Data from 1994. (A) East lobe; (B) West lobe. Different symbols represent counts from different casts.

Table 1. Growth rates measured at optimal conditions and estimated for in situ conditions for denitrifying isolates from Lake Bonney, Antarctica

			Optimal growth conditions				In situ growth conditions			
Isolate	Source	Depth of Origin (m)	Temp (°C) Optimum	Salinity (g/l) Optimum	Specific growth rate (1/h)	Gen time (h)	Temp (°C) Origin	Salinity (g/l) Origin	In situ growth rate (1/h)	Gen time (h)
ELB17	East	17	12–15	18–35	0.025	40	6	60	0.011	91
WLB20	West	20	12–15	25–50	0.024	42	−0.5	99	0.008	125
WLB35	West	35	12–15	34	0.023	43	−4	136	0	–

ing that the immunizing strains were true residents of the lake. Their growth characteristics were also indicative of organisms adapted to low temperatures. In a separate study, ELB17 was included in an analysis of diversity of denitrifying strains from various environments by restriction fragment length polymorphism (Ward, 1995) and found to cluster apart from known marine and terrestrial strains. All three Lake Bonney isolates described here appear to be psychrophilic, but their optimal temperatures exceed those found in their native environment. Similarly, the optimal salt concentrations for all three isolates were below those at the depths from which they were isolated. Salt optima in the range of 3–5% w/v (Table 1) characterizes these isolates as moderate halophiles. Using specific abundance information based on enumeration by immunofluorescence (see below), it is possible to interpret the observed distributions of the organisms *in situ* in relation to their optimal growth conditions and their growth response to different temperature and salinity conditions. IIF assays are usually very specific, and published estimates of individual strains as percent-

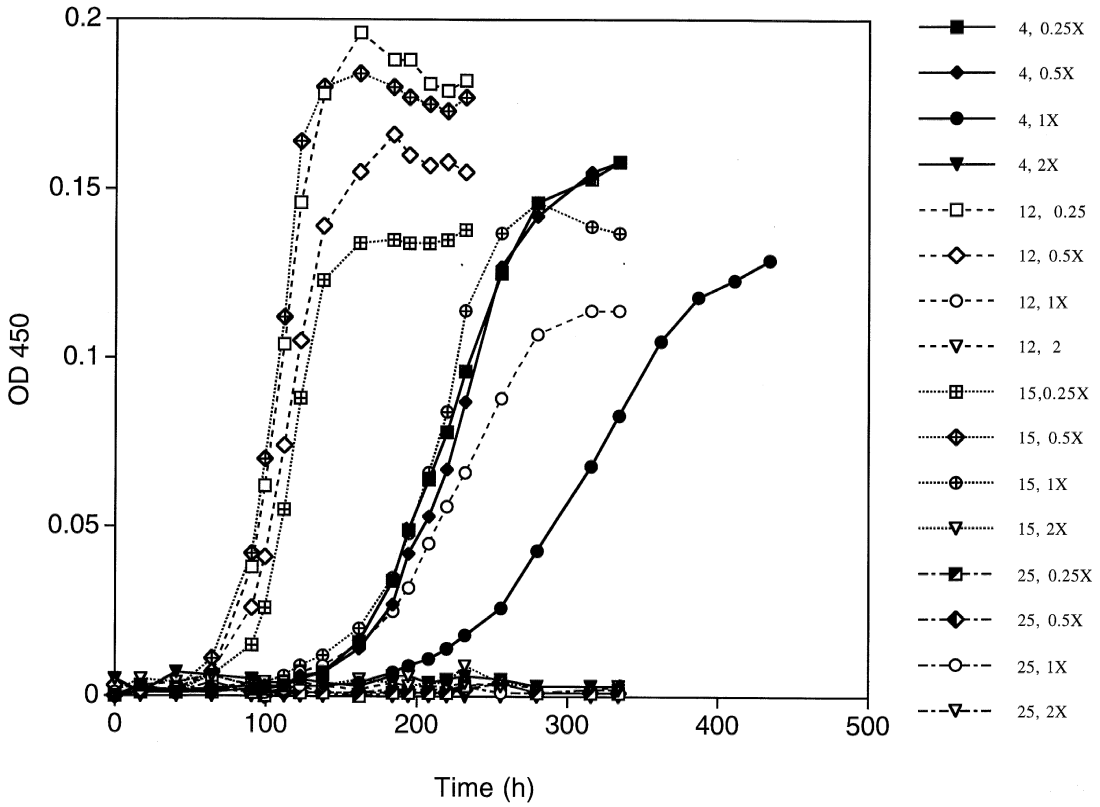

Figure 5. Growth curves for WLB20, isolate from 20 m in West lobe. Symbols represent individual flasks at 4 different temperatures and 4 different salinities. First number in flask identification refers to temperature of incubation. Second number is salinity relative to ambient level.

ages of the total population are usually in the range of less than 1%, more commonly less than 0.1% (Dahle & Laake, 1982; Ward & Carlucci, 1985; Ward & Cockcroft, 1993). Each of the two strains enumerated here by IIF represented from less than 0.01% up to nearly 5% of the total population as enumerated by AODC. ELB17 constituted 2 to 5% of the AODC total between 17 and 35 m in the east lobe and reached a maximum of about 2% at 22 m in the west lobe. These maxima occurred in the depth intervals where denitrification would be expected to occur, i.e., near the interface between oxic and anoxic layers in the water column. WLB20 represented at most 2% of the AODC total, and this was just above the chemocline in the east lobe. While WLB20 was present throughout the anoxic layer of the west lobe, it never contributed more than 0.1% of the total. These maximum abundances represent a significant portion of the total cells present, compared to the abundances of individual strains enumerated by immunofluorescence in other systems. For example, in Monterey Bay and the Southern California Bight, the abundance of *Pseudomonas stutzeri* was never greater than 0.08% and 0.2%, respectively (Ward & Cockcroft, 1993; Bard & Ward, 1997). This may mean that total species diversity in Lake Bonney may be relatively low, which is consistent with the general trend towards lower diversity in extreme environments (polar, high salinity, cold; Atlas, 1984). It is impossible, however, to assess the total number of strains present, or the total number of denitrifying strains in the lake. Therefore, we cannot estimate the extent to which these strains might represent the behavior of the total denitrifying assemblage. Nevertheless, their detection as verified by strain-specific immunofluorescence demonstrates their presence in the lake and verifies the presence of denitrifiers in both lobes of the lake. Other strains may dominate under different conditions at various depths in the water column, where ELB17 and WLB20 are minor constituents of the population.

The distribution of ELB17 was correlated with total bacterial abundance ($p \leq 0.05$, 0.05, 0.01 for the three sets of ELB17 counts) in the east lobe, and also in the

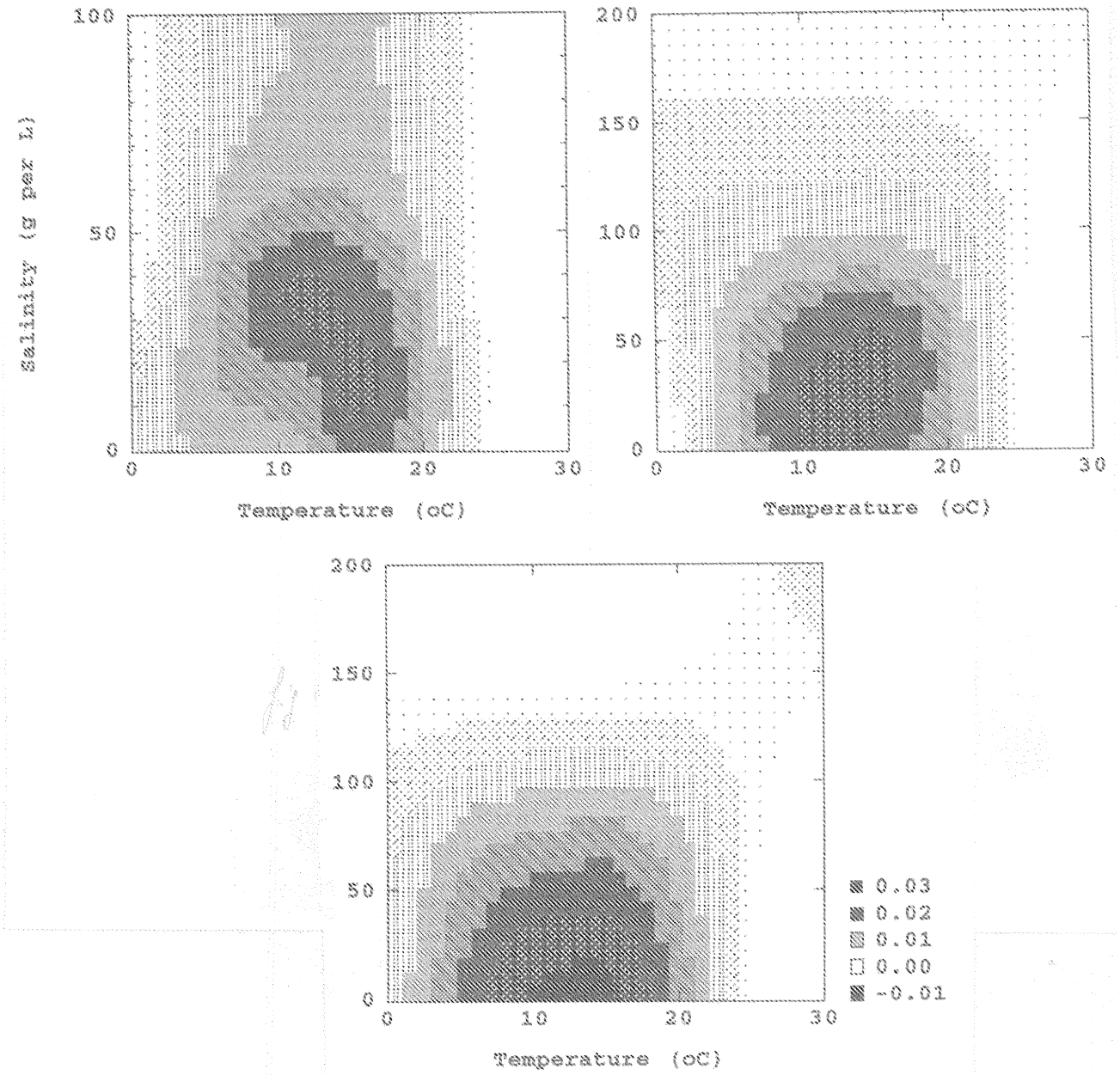

Figure 6. Variation in growth rate as a function of temperature and salinity for three denitrifying isolates from Lake Bonney. (A) ELB17; (B) WLB20; (C) WLB35. Contours were generated by SYSTAT using linear interpolation between observations. The salinity scales are different because the experimental salinities were varied according to the salinity of origin for each isolate.

west lobe ($p \leq 0.05$) while the abundance of WLB20 was not correlated ($p \geq 0.05$) with the total abundance in either lobe. The distributions of the two strains were not apparently correlated ($p \geq 0.05$) with each other within lobes. Two serotypes of nitrifying bacteria were also enumerated by immunofluorescence in this lake (unpublished data) and their distributions also did not correlate with that of the denitrifiers described here.

ELB17 originated in the east lobe at a depth of 17 m, just above the depth where its maximum abundance was subsequently detected by immunofluorescence. Sodium chloride concentration at 17 m was

about 60 g l^{-1}, nearly twice the salt concentration that allowed optimal growth. The temperature at this depth was about 6 °C. The generation time of ELB17 estimated for conditions at this depth is about 91 h. Conditions between 13 and 22 m in the west lobe where ELB17 had a broad abundance maximum, varied from approximately 2.6 °C, 90 g l^{-1} to −1.8 °C, 125 g l^{-1}. The isolate WLB20 originated within this range, at 20 m, where its generation time under *in situ* conditions is estimated to be about 125 h.

The optimal growth rates were measured, and therefore these *in situ* rate estimates were deduced, in cultures where carbon substrate was plentiful and oxygen was freely supplied. While total dissolved organic carbon levels in Lake Bonney range from about 0.2 to 1.7 mM (J. C. Priscu, unpubl. data), concentrations of labile organic substrates for bacteria, such as glucose, acetate and amino acids, are in the nanomolar to micromolar range (J. C. Priscu, unpubl. data). Growth of bacterial cultures under denitrifying conditions is generally slower than growth on the same medium in the presence of oxygen (Boogerd et al., 1984). Both carbon limitation and growth under denitrifying conditions would therefore probably reduce these growth rate estimates further. Even a 10-fold reduction in growth rate would still place these generation times within the range reported for natural bacterial populations growing at similar temperatures in the waters of McMurdo Sound (Fuhrman & Azam, 1980): minimum generation times of 53 h, and maximum generation times of thousands of hours. It seems likely, therefore, that both strains are active members of the microbial community of the lake. Even at relatively slow growth rates under *in situ* conditions, their distributions and relative abundances are consistent with the suggestion that they are actively growing at rates sufficient to maintain their presence in the lake. *In situ* loss rates are expected to be very slow due to the stable vertical stratification (near absence of sinking for bacterial sized particles), lack of turbulence and slow advection (Spigel et al., 1991) and a paucity of grazers (Priscu, pers. observ.).

Their presence in both lobes, although in different distributions, also suggests that absence of bacteria capable of denitrification is not the reason for the apparent lack of denitrification in the east lobe. ELB17 is a significant portion (up to 5%) of the total bacterial community in the east lobe where denitrification is absent, and ELB17 is just one of an unknown number of strains capable of denitrification that might be present. ELB17 is present in both lobes of the lake in distributions which suggest it is capable of persistence under conditions of the deep water in both lobes. The distribution of WLB20 implies that this strain persists at low levels throughout the water column in both lobes, and that it may be seeded into the east lobe by transport above the connecting sill depth. The fact that WLB20 occurs at very low levels below the chemocline in the east lobe suggests that the conditions of the deep water in the east lobe are prohibitive for its survival. Alternatively, the density stratification of the east lobe may be so strong as to prevent sinking and therefore seeding of the deeper layer by cells trapped in the upper layer.

If denitrifying bacteria are present in both lobes of the lake, then there must be some in situ chemical condition which inhibits their activity in one lobe versus the other. The general circulation pattern of the lake (Spigel et al., 1991) consists of input from the Taylor Glacier at the western end of the west lobe and general transport of surface water from the west into the east lobe. The distribution of WLB20 apparently reflects this circulation pattern. That is, the distribution of WLB20 is consistent with this strain being abundant in the west lobe and entering the east lobe via advection in the surface layer from west to east over the sill. Exchange of water between the lobes below sill depth is not possible due to the density stratification, but the chemocline in the west lobe is slightly shallower than in the east, so that denitrifying bacteria in the chemocline region could be continually transported from the west to the east. Data from current meter moorings in Lake Bonney corroborate this flow pattern (Priscu, unpubl. data).

Although both temperature and salinity are important determinants of growth rate in all three strains tested, simple physical parameters such as temperature and salinity are not likely to be completely inhibitory: the west lobe is colder than the east, but denitrification appears to proceed there. The east lobe is saltier, but based on their behavior in culture, the denitrifying strains investigated here should all be capable of growing actively under conditions pertaining in the region of the chemocline and even deeper. ELB17 was also present in the very salty water below the chemocline of the east lobe, although its viability there cannot be ascertained with certainty.

Although the lake is permanently stratified and Winkler oxygen measurements indicate that oxygen concentrations are low or negligible, recent electrode measurements of dissolved oxygen (Priscu, in press) indicate that the deep water of the east lobe contains

between 1 and 2 mg O_2 l^{-1} and the redox is poised in an oxidized state: Eh below the chemocline always exceeded 400 mV. Under these redox conditions, both oxygen and nitrate are thermodynamically favorable terminal electron acceptors, and denitrification may not be an advantageous respiratory mode (see also Priscu et al., 1996). In this interpretation, although denitrifying bacteria are present, they are metabolizing as aerobic heterotrophs, rather than utilizing oxidized forms of nitrogen for respiration.

Alternatively, the lack of denitrification in the east lobe may be part of a more general phenomenon of unusual bacterial activity in this lobe. Repeated attempts to measure heterotrophic bacterial production using ^{3}H-thymidine methods (Priscu, 1992) failed to detect activity in the region of the total bacterial abundance maximum below the chemocline in the east lobe. This and the accumulation of products associated with nitrification led to the hypothesis that nitrifying bacteria predominated in this region. Preliminary enumeration of nitrifying bacteria using antisera developed for marine ammonium-oxidizing bacteria did indeed detect nitrifiers in this region, but they are absent or present at very low abundances in the deep water (Voytek, Priscu & Ward, unpubl. data). It is possible that the bacterial biomass detected in the deep water of the east lobe is inactive, that the total microbial population is inhibited by the chemistry of the water, or that the cells present there are in an essentially preserved state, due to the presence of very high salt concentrations and low temperatures. Nonviable biomass might also be able to persist suspended in the water column due to the low numbers of grazers.

The summary of the recent history of Lake Bonney provided by Lyons et al. (in press) suggests a basis for the possible general inhibition of microbial life in the east lobe, or at least for the differences between the two lobes. The Taylor Valley apparently sustained a very dry period between 2000 and 2500 yr BP, causing some of the lakes to dry down and reduce volume drastically. Lyons et al. (in press) suggest that the east lobe of Lake Bonney was isolated from input from the Taylor Glacier and greatly reduced in volume but did not completely desiccate. The modern relatively fresh trophogenic surface layer of the east lobe is contiguous with and similar to that of the west lobe, but the bottom waters reflect an entirely different history. The refilling of the east lobe that began 1000–1200 yr BP has simply redissolved and diluted the salts that were concentrated in the smaller lake of the dry period. This reconstituted deep brine may be inhibitory to bacteria seeded into it from above. A closer investigation of the chemical, metal and mineral composition of this water might explain the biogeochemical differences manifested in the nitrogen and carbon cycles.

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References

Atlas, R. M., 1984. Diversity of Microbial Communities. Adv. microb. Ecol. 7: 1–47.

Bard, D. G. & B. B. Ward, 1997. A species-specific bacterial productivity method using immunomagnetic separation and radiotracer experiments. J. Microbiol. Met., in press.

Belly, R. T., B. B. Bohlool & T. D. Brock, 1973. The genus *Thermoplasma*. Ann. N.Y. Acad. Sci. 225: 94–107.

Biebl, H. & N. Pfennig, 1978. Growth yields of green sulfur bacteria in mixed cultures with sulfur and sulfate reducing bacteria. Arch. Microbiol. 117: 9–16.

Boogerd, R. C., H. W. van Verseveld, D. Torenvliet, B. Braster & A. H. Stouthamer, 1984. Reconsideration of the efficiency of energy transduction in *Paracoccus denitrificans* during growth under a variety of culture conditions. Arch. Microbiol. 139: 344–350.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Codispoti, L. A., G. E. Friederich, J. W. Murray & C. M. Sakamoto, 1991. Chemical variability in the Black Sea: Implications of continuous vertical profiles that penetrated the oxic/anoxic interface. Deep-Sea Res. 38: S69–S710.

Dahle, A. B. & M. Laake, 1982. Diversity dynamics of marine bacteria studied by immunofluorescent staining on membrane filters. Appl. envir. Microbiol. 43: 169–176.

Fuhrman, J. A. & F. Azam, 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. envir. Microbiol. 39: 1085–1095.

Lizotte, M. P., T. R. Sharp & J. C. Priscu, 1996. Phytoplankton dynamics in the stratified water column of Lake Bonney, Antarctica. I. Biomass and productivity during the winter-spring transition. Polar Biol. 16: 155–162.

Lyons, W. B., S. W. Tyler, R. A. Wharton, Jr. & D. M. McKnight, in press. The late Holocene/Paleoclimate history of the McMurdo Dry Valleys Antarctic as derived from Lacustrine isotope data. Paleogeogr. Paleoclimatol. Paleoecol.

McAuliffe, C., 1971. GC determination of solutes by multiple phase equilibration. Chem. Technol. 1: 46–51.

Parsons, T. R., Y. Maita & C. M. Lalli, 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Porter, K. G. & Y. S. Feig, 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25: 943–948.

Priscu, J. C., 1995. Phytoplankton nutrient deficiency in lakes of the McMurdo Dry Valleys, Antarctica. Freshwat. Biol. 34: 215–227.

Priscu, J. C., 1992. Particulate organic matter decomposition in the water column of Lake Bonney, Taylor Valley, Antarctica. Ant. J. US Rev. 1992: 260–262.

Priscu, J. C., 1997. The biogeochemistry of nitrous oxide in permanently ice-covered lakes of the McMurdo Dry Valleys, Antarctica. Global Ch. Biol., in press.

Priscu, J. C., B. B. Ward & M. T. Downes, 1993. Water column transformations of nitrogen in Lake Bonney, a perennially ice-covered antarctic lake. Ant. J. US Rev. 1993: 237–239.

Priscu, J. C., M. T. Downes & C. P. McKay, 1996. Extreme supersaturation of nitrous oxide in a poorly-ventilated antarctic lake. Limnol. Oceanogr., 41: 1544–1551.

Reeburgh, W. S., 1983. Rates of biogeochemical processes in anoxic sediments. Ann. Rev. Earth Planet. Sci. 11: 269–298.

Spigel, R. H., I. Forne, I. Sheppard & J. C. Priscu, 1991. Differences in temperature and conductivity between the east and west lobes of Lake Bonney: evidence for circulation within and between lobes. Ant. J. US Rev. 26: 221–222.

Spigel, R. H. & J. C. Priscu, 1996. Evolution of temperature and salt structure of Lake Bonney, a chemically stratified Antarctic lake. Hydrobiologia, 321: 177–190.

Voytek, M. A. & B. B. Ward, 1995. Detection of ammonium-oxidizing bacteria of the beta-subclass of the class Proteobacteria in aquatic samples with the PCR. Appl. envir. Microbiol. 61: 1444–1450.

Voytek, M. A., 1996. Detection, Abundance and Diversity of Aquatic Nitrifying Bacteria. Ph.D. Dissertation: 250 pp.

Ward, B. B. & A. F. Carlucci, 1985. Marine ammonia- and nitrite-oxidizing bacteria: Serological diversity determined by immunofluorescence in culture and in the environment. Appl. envir. Microbiol. 50: 194–201.

Ward, B. B. & A. R. Cockcroft, 1993. Immunofluorescence detection of denitrifying bacteria in seawater and intertidal sediment environments. Microb. Ecol. 25: 233–246.

Ward, B. B., 1995. Diversity in denitrifying bacteria: Limits of rDNA RFLP analysis and probes for the functional gene, nitrite reductase. Arch. Microbiol. 163: 167–175.