Polar Biol (1990) 10: 261–264

Polar Biology

Short Note

The Heterotrophic, Bacterial Microbiota of Burton Lake, Antarctica

P.D. Franzmann*, P.P. Deprez, A.J. McGuire, T.A. McMeekin and H.R. Burton*****

Australian Collection of Antarctic Microorganisms and Institute of Antarctic and Southern Ocean Studies, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia

Received 18 October 1988; accepted 8 August 1989

There is a paucity of information on the microbitoa indigenous to Antarctic lakes, even though the microbiota has a major influence in lake ecology and sulfur chemistry (Hand 1980). Authors often experience difficulties in the isolation of the majority of the microbiota (Lane 1977) or identification of the species present (Franzmann et al. 1987; Tearle and Richard 1987). Much of the microbiological research conducted in Antarctica is still in the "what's there?" phase of discovery. Such a study is reported for Burton Lake, a meromictic lake in the Vestfold Hills, Antarctica.

Water samples were collected at 1 m intervals with a Kammerer bottle, or, across the chemocline, at 10 cm intervals with a close interval sampler (Burke and Burton 1988). Methods for the physicochemical characterization of the water samples are detailed elsewhere (Franzmann et al. 1987, 1988). All cultivation media used were described by McGuire et al. (1987).

For determination of viable counts and subsequent isolation of representatives of the cultivatable heterotrophic bacteria of Burton Lake, 0.1 ml of each water sample was spread on the surface of a Seawater Yeast Extract Agar (SWYA) plate within 4 h of sample collection. Plates inoculated with samples collected from anoxic regions of the lake were incubated in anaerobic jars. The plates were incubated at 10°C for 14 days and different colony types were selected, purified and where possible identified to generic level by the test used by McGuire et al. (1987). Strains that grew at 10° but not at 25°C were designated psychrophilic.

Samples were examined by fluorescence microscopy (Zimmermann 1977) for the determination of total counts. Specimens deposited on polycarbonate Nuclepore filters were also examined by scanning electron microscopy (McGuire et al. 1987).

The meromictic nature of Burton Lake is exemplified in Fig. 1. The salinity of the water in the lake increased with depth from 37.3‰ beneath the ice (September 1984) to 42.9‰ at the lake bottom, 16 m (Fig. 1). The more saline and therefore more dense waters of the monimolimnion prevent mixing, although the surface waters, the mixolimnion, mix to a depth of about 12 m. The activity of the microbiota in the stagnant monimolimnion has depleted it of oxygen (Fig. 1). The accumulation of sulfide from the use of sulfate as an electron acceptor produces a drop in redox potential (Eh, Fig. 1) across the chemocline.

The occurrence of separate water bodies of distinct chemistry, and the intervening chemical gradients which result, provide niches for colonization by unique microbial communities. Numbers of bacterial rods and cocci, determined as direct counts, increased greatly below the redoxcline (Fig. 2). A secondary but minor peak in bacterial numbers occurred between 10 m and 12 m (Fig. 2) where high concentrations of bacteriochlorophyll c and numbers of *Chlorobium* spp. were present (Burke and Burton 1988). Viable counts were only in the 10^0 to 10^2 range (Table 1).

Attempts to isolate anaerobic, heterotrophic, bacterial strains from below the redoxcline on SWYA were unsuccessful. The cells were either sensitive to the brief exposure to oxygen during plating and induction of anaerobiosis in the anaerobic jars or SWYA was an unsuitable growth medium for all Burton Lake anaerobes.

A summary of the identities of the 68 bacteria isolated from the viable count plates is given in Table 2. A new taxon, *Flectobacillus glomeratus*, was described from this lake (McGuire et al. 1987) and represented 4.5% of the isolates. Fifteen percent of the strains could not be identified. Difficulties have been encountered previously with the assignation of Antarctic isolates to known taxa, and the use of ecophysiological groupings has been suggested for use in the rapid sorting of Antarctic strains (Tearle and

*Current address: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1B, D-3300 Braunschweig, Federal Republic of Germany
**CSIRO Division of Oceanography, GPO Box 1538, Hobart, Tasmania 7001, Australia
***Antarctic Division, Channel Highway, Kingston, Tasmania 7050, Australia

Table 1. Profiles for temperature, viable counts and direct counts of bacteria in Burton Lake (October 1984). Depth of redoxcline was approximately 11.3 m

Depth m	Temperature °C	Viable counts cells ml^{-1}	Direct counts		
			Rods $\times 10^6$ ml^{-1}	Cocci $\times 10^6$ ml^{-1}	Filaments $\times 10^4$ ml^{-1}
1	−1.7	30	1.3	0.36	1.2
2	−1.6	180	0.88	0.20	0.4
3	−1.6	130	0.42	0.10	1.0
4	−1.6	170	0.84	0.16	0.5
5	−1.6	170	0.84	0.16	0.5
6	−1.6	150	0.78	0.23	0.5
7	−1.6	270	0.59	0.23	1.1
8	−1.6	160	0.90	0.90	0.7
9	−1.6	130	1.1	0.16	0.3
10	−1.6	60	0.37	0.10	1.1
11	−1.5	230	1.3	0.57	4.8
12	−0.7	490	2.6	0.6	1.4
13	−0.6	0	8.1	1.4	22.9
14	−0.5	0	11.5	2.8	28.0
15	−0.4	0	19.8	12.6	48.8
16	−0.2	0	26.5	9.2	84.2

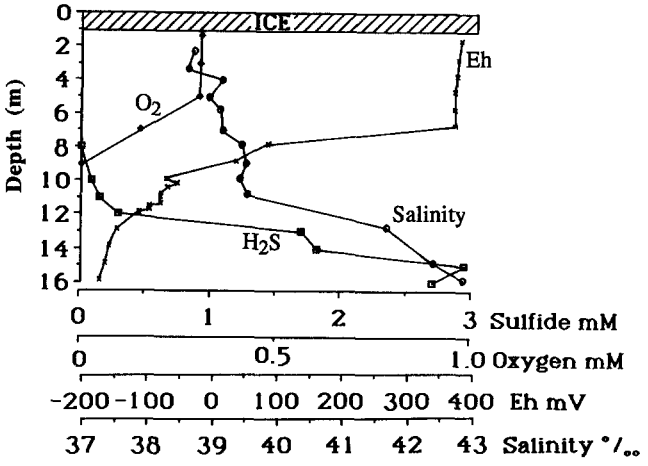


Fig. 1. Variation of salinity, hydrogen sulfide concentration, oxygen concentration, and redox potential with depth in Burton Lake (September 1984)

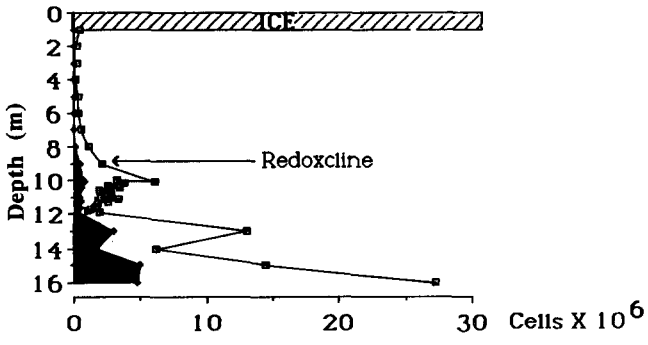


Fig. 2. Total counts of rods and cocci (SHADED) throughout Burton Lake (September 1984)

Table 2. Assignation of generic affiliations for bacterial strains isolated from heterotrophic total plate counts prepared from samples from Burton Lake

Genus	Percentage of isolates	Percentage of strains from each genus as psychrophiles
Pseudomonas	41	33
Flavobacterium	21	57
Aeromonas/Vibrio	7	20
Flectobacillus	5	100
Moraxella	5	33
Acinetobacter	1.5	0
Oceanospirillum	1.5	0
Bacillus	1.5	0
Enterobacteriaceae	1.5	0
Unidentified	15	80

Richard 1987). Adoption of such systems will create problems in relating Antarctic results to other studies in microbial ecology and taxonomy which are reliant on the binomial system for bacterial nomenclature.

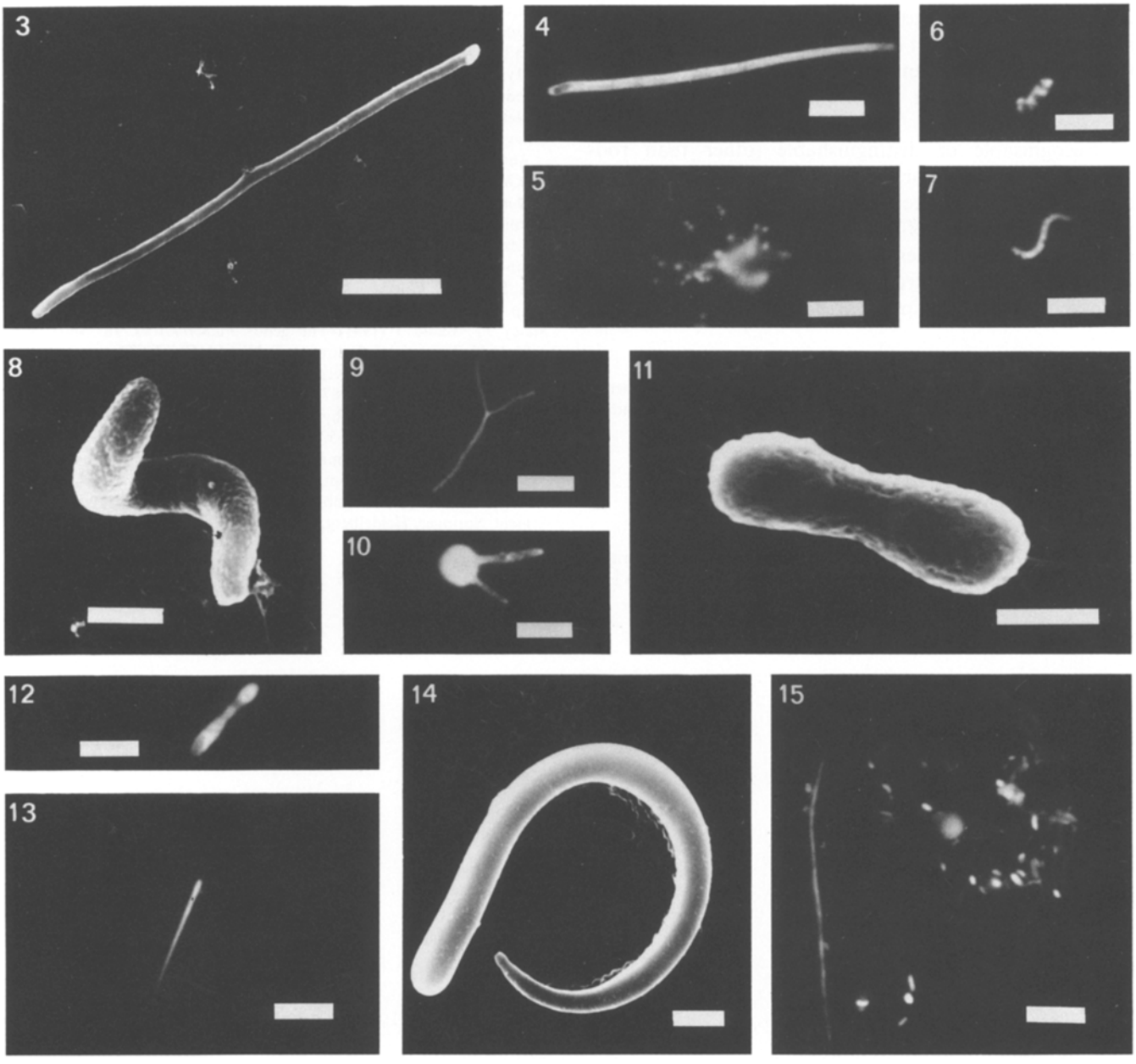
The cultivation of cells from bacterial populations in lake monimolimnia and associated chemoclines has yielded only a small proportion of the number (Table 1) and morphological types present (Hirsch 1980; Caldwell and Tiedje 1975). The distribution of a number of bacterial morphotypes was determined by scanning the filters used in fluorescence microscopy. In many cases, morphotypes were not present in the random fields used in the determination of total counts but were observed when the whole stained filter was scanned. Reliable counts of many of the unusual morphotypes at each depth were not determined but the distribution of each through the water column was recorded.

A large rod up to 60 μm long with a terminal endospore (Figs. 3 and 4), possibly a *Clostridium* sp., resided in the chemocline. It was restricted to the anaerobic zone, but did not occur below a depth of 13 m. Members of this genus produce a variety of fermentation products which could serve as electron donors for sulfate reduction or anoxygenic photosynthesis.

Cells which bud and attach to detritus or other cells by a secreted stalk (*Planctomyces* spp.) (Fig. 5) were observed twice and only from water collected from the anoxic monimolimnion. This morphotype occurred in water with oxidation–reduction potentials between − 75 and − 90 mV, and in sulfide concentrations less than 18 $mg\,l^{-1}$. All members of the genus which have been cultivated are aerobic chemoheterotrophs (Staley and Bauld 1981), thus it is possible the morphotype does not grow at the depth from which it was drawn.

A thick, cigar-shaped cell displayed banding when viewed by fluorescence microscopy after staining acridine orange (Fig. 6). It was restricted to the aerobic mixolimnion.

Spirilla (Figs. 7 and 8) were common throughout the water column, averaging about 4×10^4 cells ml^{-1} in the mixolimnion and about twice that density in the



Figs. 3–15. Scanning electron micrographs (**3, 8, 11, 14**) of Burton Lake morphotypes and photomicrographs of acridine orange stained cells illuminated with ultra violet radiation (**4, 5, 6, 7, 9, 10, 12, 13, 15**). Cells shown include: endospore former (**3, 4**): *Planctomyces* sp. (**5**); cigar-shaped morphotype (**6**); spirilla (**7, 8**): actinomycete (**9**): arthrospore (**10**): "dumbell" morphotype (**11, 12**); "needle" morphotype (**13, 14**); sheathed filament and a choanoflagellate, *Diaphoneca spherica* (**15**). [Bar = 10 μm in **3, 4**; 5 μm in **5, 6, 7, 9, 10, 12, 13, 15**); 1 μm in **8, 11, 14**]

monimolimnion. High concentrations occurred immediately beneath the oxycline (22×10^4 cells ml^{-1} at 11.6 m in October) where *Thiospira* spp., which are chemotactic with respect to O_2 and H_2S, would be expected to aggregate (la, Rivière and Schmidt 1981).

A branching morphotype (Fig. 9) was encountered on eight occasions between depths 7 to 11.4 m in October and therefore colonizes aerobic to microaerophilic niches. Its presence coincided with the presence of what appeared to be a germinating arthrospore (Fig. 10). Cells of this morphotype could be representatives of a number of genera of coryneform bacteria.

Other unusual morphotypes include a large dumbbell-shaped cell (Figs. 11 and 12) which occurred in the anoxic zone within the chemocline; an aerobic needle-shaped rod, rounded at one end and tapered at the other (Figs. 13 and 14), and an aerobic filamentous cell which is sheathed, shown in Fig. 15 with the choanoflagellate *Parvicorbicula socialis.*

Recognisable or distinguishable (other than rod-shaped or coccid) morphotypes throughout the water column in Burton Lake were few in number when compared to the number of morphotypes recognised in non-Antarctic stratified lakes. It is possible that fewer species inhabit the colder Antarctic waters in which psychrophilic or psychrotrophic adaptations would promote colonization.

Acknowledgements. We thank the Australian Research Grants Scheme, the University of Tasmania and the Antarctic Division for their funding and logistic support of this project.

References

Burke CM, Burton HR (1988) The ecology of photosynthetic bacteria in Burton Lake, Antarctica. In: Ferris JR, Johnstone GW, Burton HR, Bayly IAE (eds) Proc Symp Biol Vestfold Hills, Antarctica. Kluwer Academic Publications, Dordrecht, Netherlands, pp 1–11

Caldwell DE, Tiedje JM (1975) A morphological study of anaerobic bacteria from the hypolimnia of two Michigan lakes. Can J Microbiol 21:362–376

Franzmann PD, Deprez PP, Burton HR, van den Hoff J (1987) Limnology of Organic Lake, Antarctica, a meromictic lake that contains high concentrations of dimethyl sulfide. Aust J Mar Freshw Res 38:409–417

Franzmann PD, Skyring GW, Burton HR, Deprez PP (1988) Sulfate reduction rates and some aspects of the limnology of four lakes and a fjord in the Vestfold Hills, Antarctica. In: Ferris JR, Johnstone GW, Burton HR, Bayly IAE (eds) Proc Symp Biol Vestfold Hills, Antarctica. Kluwer Academic Publications, Dordrecht, The Netherlands, pp 25–33

Hand RM (1980) Bacterial populations of two saline Antarctic lakes. In: Trudinger PA, Walter MR (eds) Biochemistry of ancient and modern environments. Australian Academy of Science, Canberra, pp 123–129

Hirsch P (1980) Distribution and pure culture studies of morphologically distinct Solar Lake microorganisms. In: Nissenbaum A (ed) Hypersaline brines and evaporative environments. Developments in sedimentology, vol 28. Proc Bat Sheva Seminar Saline Lakes and Natural Brines. Elsevier, Amsterdam, pp 41–60

Lane LS (1977) Microbial community fluctuations in a meromictic Antarctic lake, Hydrobiologia 22:187–190

McGuire AJ, Franzmann PD, McMeekin TA (1987) *Flectobacillus glomeratus* sp. nov. a curved nonmotile pigmented bacterium isolated from Antarctic marine environments. Syst Appl Microbiol 9:265–272

Rivière JWM la, Schmidt K (1981) Morphologically conspicuous sulfur-oxidizing eubacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes. Springer, Berlin, Heidelberg, pp 1037–1048

Staley JT, Bauld J (1981) The genus *Planctomyces.* In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes. Springer, Berlin, Heidelberg, pp 503–508

Tearle PV, Richard KJ (1987) Ecophysiological grouping of Antarctic environmental bacteria by API2ONE and fatty acid fingerprints. J Appl Bacteriol 63:497–503

Zimmermann R (1977) Estimation of bacterial number and biomass by epifluorescence microscopy and scanning electron microscopy. In: Reinheimer G (ed) Microbial ecology of a brackish water environment. Springer, Heidelberg, pp 103–120