

ABUNDANCE, VIABILITY AND CULTURABILITY OF ANTARCTIC BACTERIA

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Abstract

The viability of total number of bacteria decide the mineralisation rate in any ecosystem and ultimately the fertility of the region. This study aims at establishing the extent of viability in the standing stock of the Antarctic bacterial population in different niches besides estimating their retrievable numbers as colony forming units (CFU). It is also for the first time that retrievable anaerobic groups have been enumerated and the viability of the potential fraction capable of growing in reduced/anoxic conditions deciphered. About 0.01-1.0% of the flora have been retrieved either as aerobic or anaerobic CFUs. The total number as estimated by the AODC (Acridine Orange Director Counts) showed that it varied from 2.62×10^8 to 4.21×10^{10} in lakes and from 4.3 to 8.51×10^{10} l in sea water. The anaerobic retrievability in the lakes was sometimes more by 2 orders. The mean percentages of viability in different lakes varied from 4.3 to 64.5% under aerobic and from 6.4 to 99.3% under anaerobic conditions. The average percentage of viability in sea water was maximum at 5 m (58.5%) under aerobic conditions, and at 50m under anaerobic conditions (78.2%). The percentage viability in the different niches suggest that the bacterial population could be active in the turnover of matter. This could particularly be true in some of the lakes and sub-surface oceanic waters where not only the bacterial standing stock is high but also the viable forms. As growth under anaerobic conditions is generally lower it is suggested that the propensity for higher viability and retrievability under anaerobic conditions could be a strategy for survival exhibited by the bacterial flora to overcome the double stress of low temperature and oxygen supersaturation in the Antarctic fresh waters.

Introduction

Microbes especially bacteria form an important component of any ecosystem and they contribute towards its steady state by then twin fold as mineralisers and producers. Microheterotrophs are crucial for understanding the food web systems and this aspect does not fall into the classical rhythm of bacterial population following the phytoplankton in the Antarctic ecosystem. This uncoupling has sometimes shown to be marked in this region (Rivkin *et al*, 1991). Karl *et al* (1991) have also shown that the bacterial numbers did not vary

systematically during the 4-month period of observation and thus appear to be uncoupled from phytoplankton dynamics. Others have stressed that bacterioplankton is an important component of marine pelagic ecosystems and their production is an important sink for organic material in most of the ecosystems including the Antarctic. However, recently questions have been raised about the significance of bacterioplankton production and carbon fluxes in polar waters (Hollibaugh *et al.*, 1992). Nedwell and Rutter (1994) suggest that there are decreased affinities for substrates by heterotrophic bacteria in polar seas due to decreased affinity of substrate uptake at low temperatures. On the other hand there are reports which point out to their active participation (Kogure *et al.*, 1986). Thus while the total number of bacteria obtained by epifluorescence microscopic counts give the standing stock, an assessment of their viability would reflect their ability to degrade and participate in various activities at their disposition in their niche. Keeping this in view an attempt was made to estimate the potentially viable fraction versus the total bacterial number under the Indian Antarctic Programme.

Though the study of Antarctic microbiology dates back to the sixties, the Indian expedition to Antarctica was initiated for the first time in 1998. Although a decade has passed, the research in this field has been sporadic and limited (Matondkar and Gomes, 1983; Shivaji, 1987; Ramaiah *et al.*, 1994; Ramaiah, 1995). This study aims at determining the viability of the Antarctic bacterial flora for the first time and it has also examined the viability of the potential anaerobes. The retrievable number of anaerobes like the general organotrophs i.e. the anaerobic bacteria (AnB), fermentative bacteria (FB), sulfate reducing bacteria (SRB) and *Thiobacillus denitrificans* like organisms (TDLO) have also been examined for the first time in this realm during the summer logistics seasons.

Materials and Methods

During the 13th-Antarctic expedition (Dec 1993-March 1994) microbiological sampling was carried out at 28 stations. Among these, 10 stations were along the cruise track from Dakshin Gangotri (First Indian Base Camp) to Durban Port (**Fig.1**). Sea water was sampled from depths of 5, 50 and 100 m using a ZoBell's sampler. Samples from the continent included water, soil and ice samples. Surface waters from and around Maitri lake (**Fig.2**) were collected using sterile flasks. The bottom waters from lake Maitri (**Fig.2**) were collected using Niskin's samplers. Samples collected in and around the Schirmacher Oasis of the Queen Maud Land included 44 freshwater samples, 19 sediment samples, 14 vegetative and 3 samples from a penguin rookery. Water and ice

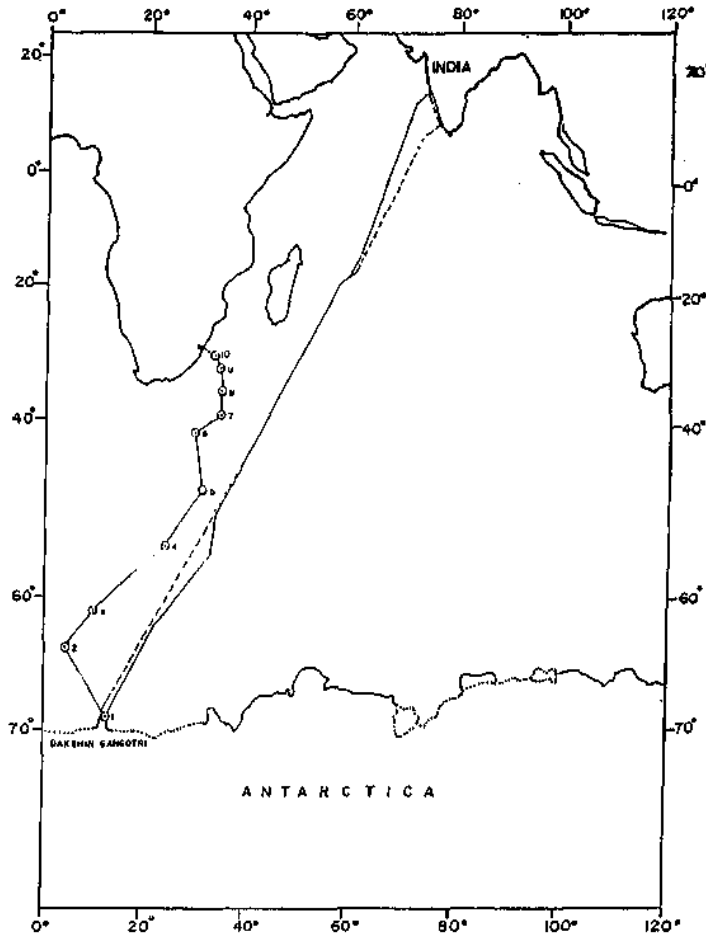


Fig. 1: Sampling stations from Dakshin Gangotri to Durban

samples were suitably preserved with 2% formalin until they were enumerated for total counts (TC) using AODC method (Hobbie *et al.*, 1977). The bacterial numbers were estimated using epifluorescence microscopy. Quantitative enumeration of the total viable aerobic (TVC) and anaerobic (TVAn) population was done as outlined by Kogure *et al.* (1980) using nalidixic acid and yeast extract. However, for determining the viability under anaerobic conditions a reductant i.e. Na_2S at a final concentration of 0.125% was added before incubating the samples. These experiments were carried out in screw capped tubes filled to the brim to minimise oxidation. As the tubes were incubated under low ambient temperatures of 8-12°C, the period of incubation was

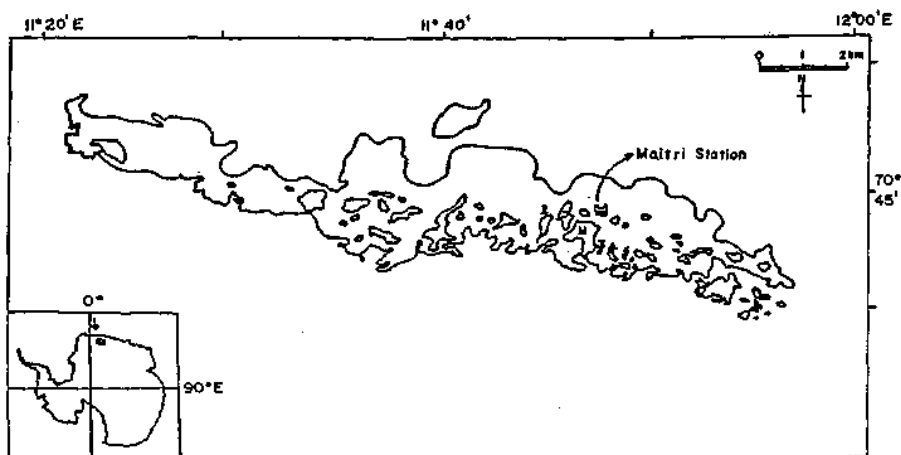


Fig. 2: Lakes around Maitri Station in the Schirmacher Oasis

extended to 12-16 hrs for samples from and near Antarctica. For retrievable number, colony forming units (CFU) were counted on suitable media by spread plating. Plating was carried out within a few hours of collection. Sea water nutrient agar and ZoBell's marine agar were used for enumeration of marine bacterial populations. Nutrient agar prepared with freshwater was used to estimate the CFU from limnetic and terrestrial samples. Samples were serially diluted and spread plated for aerobic counts (AB) and inoculated in agar shake tubes for anaerobic counts (AnB) (Loka Bharathi, 1989). The final volume of inoculum in the tube being as high as 5 ml in 15 ml screw capped tubes and the concentration of agar only 0.8%, heat shock to the microbes was minimal. Likewise modified Hatchikian's (Loka Bharathi and Chandramohan, 1990) and Leiske's medium (Loka Bharathi, 1989) were used for enumerating SRB, FB and TDLO, respectively. All colonies that were not black by sulfide precipitation were counter as fermentative bacteria (FB). The anaerobic CFU were AnB (Anaerobic bacteria), FB (Fermentative Bacteria), SRB (Sulfate reducing bacteria), and TDLO (*Thiobacillus denitrificans* like organisms). Plates and tubes were incubated between 8 to 12°C for 2 weeks and the CFU were counted.

As the sampling covered different types of ecosystems, they have been grouped together under different heads. Thus all samples in and around Maitri lake (Maitri is the Second Indian Base Camp) are grouped together. The next cluster deals with those lakes about 2 km east and west of Maitri. The rest of the data are dealt as per their origin i.e., their retrievable numbers from the soil, vegetation and other miscellaneous sources.

Results

Sea water samples showed interesting trends. The range in TC was from 5.9×10^8 to $2.1 \times 10^{10} \text{ r}^{-1}$ at 5 m depth, 4.37×10^8 to $8.51 \times 10^{10} \text{ l}^{-1}$ at 50 m and 4.47×10^8 to $2.37 \times 10^{10} \text{ l}^{-1}$ at 100 m. The mean populations of 1.17×10^{10} was highest at 50 m. The mean percentage of anaerobic viability at 78.2% was also the highest at this depth. The average percentage of viability of aerobes decreased with depth from 58.31% at 5 m to 40.5% at 100 m. The average numbers of SRB were 0.01, 0.04 and $0.13 \times 10^3 \text{ l}^{-1}$ in these depths. The corresponding average TDLO numbers were 0.14, 0.2 and $0.19 \times 10^3 \text{ l}^{-1}$ (**Table-1**). The range of total count in the Maitri Lake was from 5.9×10^8 to $1.35 \times 10^{10} \text{ l}^{-1}$ with average of $23.6 \times 10^8 (\pm 35.71)$. Total aerobic viable counts were 2 orders magnitude lower than total counts. The viable counts were more pronounced under anaerobic conditions than under aerobic. The CFU of AB were 10^3 l^{-1} with AnB having the same trend as the TVC. SRB were retrieved less frequently and occurred at 0.1 to $0.2 \times 10^4 \text{ l}^{-1}$ range and were detected in both surface and bottom samples. They were absent along the coast of the lake except at one side (**Table-2**). Although the total bacterial population of the lakes at the vicinity of Maitri (east & west) was less, the total viable anaerobes were more dominant than in lake Maitri. The lakes 2 km away from Maitri both on the eastern and western side did not show spatial difference in bacterial abundance (**Table-3**). However the lakes situated on the eastern side had a lower bacterial load than on the western side. The retrieval number from all the five samples of the western lake was almost equal and the AB load was as low as 10^1 l^{-1} . In the lakes that are spread to the west of Maitri, the difference in maximum and minimum was only 10 times. In general the Antarctic lake ecosystem had a bacterial load ranging from 10^8 to 10^{10} l^{-1} which had a viability ranging from 0.01 to 99.2% under aerobic and from 17.3 to 100% under anaerobic conditions (**Table-4**).

In the soil the number of AB ranged from non-detectable level to $1.94 \times 10^7 \text{ gm}^{-1}$. The AnB were about 5 orders less where as SRBs were hardly detected. The frequent occurrence of TDLO was enigmatic. In the vegetation sample a maximum number of bacteria of $1.49 \times 10^7 \text{ gm}^{-1}$ was recorded. Very high retrieval number upto 10^{10} gm^{-1} was natural in the rookery sample whereas in the ice it was as low as $0.4 \times 10^2 \text{ ml}^{-1}$. In the rookery, the TDLO, SRB and FB were well represented (**Tables 4-6**).

Discussion

Azam *et al.* (1991) indicate there is a paradigm shift in pelagic food web organisation in Antarctic waters in that a large fraction of energy and material flow through the microbial loop. While TC give the bacterial standing stock, TVC give an estimate of the total viable i.e. the active flora. Kogure *et al.* (1984) advocate the use of 3 antibiotics viz. nalidixic, piromedic and pipemedic acids to ensure sufficient enlarging of cells by longer incubation. They have also shown that there has not been significant difference in the counts by nalidixic

Table-1: Total, viable and retrievable number of bacteria from Sea Water Samples

Depth (metre)	Station No.	No x		10 ⁸	l ⁻¹	ICFU _x		10 ⁴ l ⁻¹		T D L O
		TC	TVC	TVan	AB	AnB	FB	SRB		
5	1	11.13	6.87	2.5	nd	0.004	0.1	nd	0.1	
	2	16.37	6.77	2.0	0.001	0.002	nd	nd	0.1	
	3	-14.19	5.89	2.0	5.6	0.004	nd	nd	nd	
	4	10.0	5.89	2.5	2.4	0.006	0.1	nd	0.5	
	5	16.15	10.0	2.0	0.15	0.002	nd	nd	nd	
	6	10.2	10.19	9.6	47.2	0.06	0.5	nd	0.1	
	7	7.2	7.1	7.2	10.2	0.016	0.2	nd	nd	
	8	8.29	4.8	2.5	53.2	0.024	0.1	0.1	nd	
	9	191.19	8.29	2.0	16.2	0.01	0.3	nd	0.3	
	10	199.33	18.96	12.4	0.32	0.022	0.4	nd	0.3	
50	1	4.37	2.4	4.37	0.2	0.006	nd	nd	0.3	
	2	13.97	2.2	9.17	4.4	nd	nd	0.2	nd	
	3	8.51	2.5	8.51	0.4	0.004	nd	nd	1.1	
	4	13.06	2.0	12.16	2.6	0.036	nd	0.1	nd	
	5	7.64	2.0	7.64	0.01	0.01	nd	nd	nd	
	6	35.0	34.7	8.73	24.0	0.02	nd	0.1	nd	
	7	21.0	20.5	7.42	10.6	0.01	0.44	nd	nd	
	8	5.46	2.6	5.46	15.0	0.006	0.8	nd	nd	
	9	209.52	2.0	131.09	6.20	0.004	0.1	nd	0.6	
	10	851.18	840.3	851.18	1.14	0.022	0.1	nd	nd	
100	1	4.47	4.4	2.5	0.8	0.012	0.1	nd	1.5	
	2	25.32	2.5	17.46	13.0	nd	nd	nd	nd	
	3	12.22	5.7	12.22	0.8	0.002	0.1	nd	nd	
	4	11.57	5.9	2.62	96.0	nd	nd	0.2	nd	
	5	20.08	2.0	6.55	0.53	0.03	nd	nd	nd	
	6	11.0	10.7	11.0	14.0	0.02	nd	nd	nd	
	7	5.67	2.5	5.67	18.0	0.018	nd	0.1	nd	
	8	237.46	2.0	66.78	8.40	0.01	nd	nd	nd	
	9	23.64	2.0	23.64	7.8	0.006	0.1	nd	0.4	
	10	77.04	28.36	6.11	0.9	0.012	nd	1.0	nd	

(nd : not detected)

Table-2 : Total, viable and retrievable number of bacteria in and around Maitri Lake

Source	No		x		$10^8 l^{-1}$		$CFU \times 10^4 l^{-1}$	
	TC	TVC	TVan	AB	AnB	FB	SRB	TDLO
Surface	9.2	0.01	9.17	0.002	0.2	0.2	nd	0.2
(4)	6.3	1.8	6.22	0.062	9.0	0.2	nd	0.2
	135.0	6.4	134.22	0.12	13.4	3.2	0.2	0.2
	10.3	0.7	10.26	0.006	16.2	1.4	nd	0.2
Bottom	8.7	0.01	8.7	0.002	26.2	2.8	0.2	0.4
(4)	8.3	0.01	5.46	0.032	20.4	0.2	nd	0.6
	13.2	2.20	8.51	0.002	101.8	3.0	nd	4.0
	6.6	0.01	6.55	0.060	22.0	0.6	0.2	0.8
Coastal	9.9	0.3	9.93	0.146	3.2	0.8	2.4	5.0
(6)	15.1	0.01	10.26	0.166	1.4	0.8	0.2	nd
	67.9	0.01	56.09	0.088	1.6	1.6	0.4	nd
	16.81	1.3	16.81	0.448	2.2	0.4	nd	nd
	16.81	0.3	16.81	0.052	5.6	0.4	0.2	nd
	5.9	1.2	5.89	0.056	3.6	1.4	nd	nd
Lakes	11.46	5.2	6.33	1.8	0.8	0.6	0.6	3.6
East &	23.46	2.8	4.04	1.01	0.8	3.6	1.2	100.0
West of	10.69	2.5	10.6	0.02	0.4	1.2	0.6	0.2
Maitri	4.15	2.5	4.15	0.03	0.8	nd	nd	0.4
(6)	4.58	2.0	2.5	0.01	1.4	0.4	nd	1.6
	7.75	2.7	7.75	0.17	1.6	nd	nd	1.2

(nd : not detected; 0 number of samples)

Table-3 : Total, viable and retrievable numbers of bacteria in lakes 2 km East and West of Maitri

Area	No		x		$10^8 l^{-1}$		$CFU \times 10^4 l^{-1}$	
	TC	TVC	TVan	AB	AnB	FB	SRB	TDLO
East	4.8	2.5	2.62	0.02	2.2	0.2	nd	0.2
Lake 1	2.84	2.5	2.5	0.004	0.8	nd	nd	nd
(5)	6.87	2.5	3.93	0.004	0.2	0.2	nd	nd
	3.93	2.0	2.5	0.014	nd	0.2	nd	nd
	2.62	2.5	2.5	0.002	1.0	1.0	nd	4.6
West	32.0	31.7	16.7	0.548	8.80	nd	nd	3.6
Lake 1	146.23	39.7	109.56	0.142	2.60	nd	nd	0.2
(6)	68.53	46.7	52.6	0.03	0.20	nd	nd	nd
	167.5	35.1	167.4	0.016	0.20	nd	nd	0.2
	296.82	16.2	278.27	0.040	2.60	nd	nd	0.2
	77.0	76.4	13.31	0.062	0.20	nd	nd	nd

(Contd.)

Table 3—Contd.

Lake 2	421.49	21.8	213.89	0.044	0.20	nd	nd	nd
(4)	21.0	19.6	20.01	0.032	1.0	nd	nd	nd
	20.19	16.2	18.66	0.126	64.0	nd	nd	nd
	16.0	8.0	16.0	0.246	5.4	0.6	nd	1.8
Lake 3	13.2	0.01	13.20	0.038	8.6	nd	nd	5.2
(5)	76.17	23.4	76.17	0.062	52.0	nd	0.4	1.8
	130.4	0.9	97.45	0.012	3.0	1.4	nd	0.6
	67.55	16.0	28.48	0.064	1.2	0.4	nd	0.4
	47.47	5.30	31.46	0.490	3.2	0.4	nd	0.4
Lake 4	161.49	0.01	64.06	0.022	4.4	0.2	nd	0.6
(4)	7.86	0.01	6.33	0.014	14	0.2	nd	1.0
	6.56	1.40	6.56	0.066	9.2	nd	nd	0.2
	15.2	4.20	7.75	0.022	0.4	0.8	nd	0.8

(nd : not detected)

Table-4 : Retrievable number from Oasis/Dry valley Soil ($\times 10^5 \text{g}^{-1}$)

Area	AB	AnB	FB	SRB	TDLO
I(13)	0.006	0.004	0.004	nd	0.001
	0.074	0.0004	0.0004	nd	nd
	0.091	0.001	0.0001	0.001	0.013
	0.240	0.066	0.066	nd	0.009
	nd	0.045	0.045	nd	0.005
	nd	0.006	0.006	0.020	0.490
	0.050	0.003	0.003	nd	0.003
	23.170	0.077	0.077	0.042	0.003
	nd	0.006	0.066	nd	nd
	2.180	nd	nd	nd	0.002
	0.880	0.009	0.009	0.002	nd
	7.680	0.01	0.010	0.007	0.130
	2.480	0.04	0.004	0.006	0.031
II (1)	5.400	0.05	0.005	0.001	0.003
III (2)	nd	0.024	0.024	nd	nd
	nd	0.023	0.023	nd	nd
IV (3)	0.190	0.002	0.002	nd	0.006
	7.790	0.13	0.013	nd	0.002
	194.2	0.003	0.003	nd	0.008

(nd : not detected)

Table-5 : Retrievable number from Antarctic Vegetation ($\times 10^5/\text{g}^{-1}$)

Area	Source	AB	AnB	FB	SRB	TDLO
I(7)	Blue green moss	nd	0.004	0.018	nd	1.770
	Black moss	nd	0.012	0.00041	nd	0.130
	Dried lichen	149.52	0.016	0.032	nd	0.040
	Mossy sand	4.09	0.006	0.006	nd	0.040
	Black moss	1.03	0.010	0.003	0.002	0.009
	Yellow Green moss	4.02	0.008	0.008	0.002	0.009
	Dry moss	56.91	0.007	0.004	nd	0.190
II (3)	Green moss	1.57	0.016	0.003	0.002	0.11
	Mossy sand	74.21	0.004	0.004	0.005	0.009
	Mossy sand	4.79	0.007	0.002	nd	0.010
III (4)	Dry moss	2.80	0.180	0.060	nd	0.150
	Moss and algae below ice	46.21	0.020	0.100	0.210	0.160
	Moss	118.22	0.010	0.023	nd	0.020
	White encrusted algae	nd	0.002	nd	nd	0.002

(nd; not detected)

Table-6 : Retrievable number from Miscellaneous Sources ($\times 10^5 \text{ g}^{-1}$ or ml^{-1})

Area	Source	AB	AnB	FB	SRB	TDLO
I(1)	Ice	0.004	nd	nd	nd	nd
II(3)	Rookery sand	1518.7	0.62	5.73	0.25	1.74
	Rookery egg shell	79852.94	2.05	3.72	2.94	2.94
	Rookery stone	17022.22	0.89	6.22	0.22	0.860
III(1)	Petrel bone	17813.410	0.33	nd	nd	nd
IV (2)	Brown ice	nd	0.008	nd	nd	0.020
	Brown ice	nd	0.02	0.006	0.01	0.010

(nd : not detected)

acid alone and in combination with the other acids. Hence in the present study only nalidixic acid has been used and the period of incubation increased upto 16 hrs for the above reasons. Moreover, incubation under colder ambient temperatures of 8-12 °C also necessitated long incubation for notable results. Addition of Na_2S ensured sufficient anoxia.

In the Antarctic, despite low water temperatures, bacteria play an important role in the energy transfer within the southern oceanic ecosystem. The number of counts in the sea water was high and ranged from 10^8 to 10^{10}l^{-1} . It is higher by two orders than that reported by Zdanowski and Donachie (1993). The AODC counts by these authors in the sea water in the summer of 1988/89 was $6.0 \times 10^6\text{l}^{-1}$. Our values for AB are also two fold higher than 10^3l^{-1} range reported by them. The other reports (Kim, 1991) in the range 10^4 - 10^5 cells ml^{-1}

are also lower than the present values. However, seasonal fluctuations in bacterial numbers near the Antarctic continent showed that there was a significant decrease in their numbers in early December and then recovered later in the month at the end of an algal bloom. The highest number of cells reported in this case was $>1 \times 10^6$ cells ml^{-1} (Gibson *et al.*, 1990). Bacteria with their large mineralisation rates of amino acids and their low sinking rates, appear to be responsible for a large portion of organic matter recycling in the upper surface water of the coastal Antarctic ecosystem (Tupas *et al.*, 1994). In spite of the above fact, high bacterial density was not restricted to the surface waters in our study. Though the mean viability of 48.5% at 50 m was lower than the surface mean of 58.3% the high anaerobic viability of 78.2% at 50 m point out to a high mineralisation potential at this depth. Increased viability and *retrieve* ability under anoxic conditions could perhaps indicate their adaptability to the low temperature stress. Viability could be prolonged by the reduced growth rates under anaerobic or reduced conditions as opposed to faster rates Under oxic conditions. The highest number of bacterial cells are often observed in the stratum containing the dissolved carbon matter or primary production maximum. Thus relatively large abundance and biomass of bacterioplankton and the high percentage of viability suggest that even in the southernmost open areas of the Antarctic zone, micro-organisms are involved in the recycling of nutrients i.e. a considerable fraction of matter and energy flows through the microzooplanktonic community that depend on them for nutrients (Sazhin, 1993)•

A wide range of bacterial heterotrophs have been identified in Antarctic lakes and there is no evidence till date that the polar environment substantially limits the distribution and activity of the microbial group. The bacterial counts as estimated by direct epifluorescence reveal similar concentrations in the Antarctic lakes to those observed in the temperate latitudes i.e. in the $10^5 - 10^6$ ml^{-1} range (Vincent, 1988). Our observations reveal the range 10^8 to 10^{10} ml^{-1} in which the maximum is one order higher. In the lakes extending to the west of Maitri the range was from 6.56×10^8 to 4.21×10^{10} with an average of 9.44×10^9 ml^{-1} . The propensity of anaerobic growth was generally high and ranged from 17.3 to 100%. Though the retrieval of aerobic flora was in $<10^2$ ml^{-1} range, the anaerobes could be more frequently enumerated in higher numbers. The average viability was much higher (71.4%) among the anaerobes than among the aerobes (31.2%). Perhaps this is an overestimate of the viability as pointed out by Kogure *et al.*, (1984). Nevertheless the method gives an insight into their potential activity. The viability could also be attributed to prevailing conditions. Some of the estimated growth rates in the regions of the ice edge fell in the range $0.001-0.45$ d^{-1} (mean 0.09 d^{-1}) in the southern ocean (Vincent, 1988).

The inclination to exhibit viability under anaerobic conditions in bacteria from realms of high concentration of oxygen is quite intriguing. The fresh water lakes of Schirmacher Oasis are known to contain dissolved oxygen in the range 10.4-13.8 mg l⁻¹ (Ingole and Parulekar, 1993). Besides, it is known that freezing in ice covered lake's causes oxygen supersaturation in water (Craig *et al*, 1992; Webster *et al*, 1996). Hence it is hypothesised that these microbes have developed the tendency to grow better under reduced conditions not only to overcome the low temperature stress but also to bypass the negative influence of oxygen supersaturation in Antarctic fresh waters. Apparently the low temperature could be stressful even to Antarctic microbes because it has been often emphasised that a large majority of Antarctic bacterial strains must be considered psychotrophic and not as true psychrophiles (Inone and Komagata, 1976; Delille and Perret, 1989), the former being more ecologically competitive than the latter.

SRB were less frequently encountered. Perhaps they were limited in distribution not only due to the absence of suitable substrates but also electron acceptors like sulfate and other oxidised sulfur compounds in the lakes. Sulfate reduction has been noticed in lakes receiving inputs of marine derived salts (Vincent, 1988). However, De Long *et al* (1994) have reported that as high as 34% of the prokaryotic biomass was composed of Archaea. The numbers in this study could indicate low sea water ingress. It is also possible that several of the SRB were in a viable but non culturable state and therefore could not be retrieved (Colwell *et al*, 1987). Besides, Konda *et al* (1994) have found that while TC increased with increasing depth from 10⁵ to 10⁷ cells ml⁻¹ in lakes SRB could be retrieved only from the bottom layers. TDLO were more frequently encountered than SRB. The disparity in numbers between these two groups was much higher than in Maitri. It is possible that the latter receive their electron donors in the form of reduced sulfur compounds from products of bio-degradation rather than from sulfate reduction.

Despite high bacterial biomass in Antarctic sediments the rate of bacterial DNA synthesis was some 300 times slower than in estuarine sediments at lower latitudes. The average growth rate measured with tritiated thymidine was 7.6 x 10⁻³ cell divisions hr⁻¹ g⁻¹ sediment (Vincent, 1988). Moreover the lipid profiles of sediment have been found to be different from the overlying waters and there is an indication of extensive bacterial degradation and recycling of labile lipids (Volkman *et al*, 1988). It has been observed that surficial sediments of Antarctic lakes contain orders of magnitude higher concentration of bacteria than that of the overlying water column (Vincent, 1988). The total counts, retrievable counts in the form of both aerobic and anaerobic CFU, and even viability have a different trend in the soil. The retrieval of AB>AnB and other

anaerobes in soil and vegetation is usually the normal trend. Soil desiccation may perhaps override the stress of low temperature. Here, the flora need not force their propensity to grow anaerobically to be viable because the lack of moisture would make them totally dormant. The retrieval of all groups of bacteria in high numbers in the rookery samples indicate a rich niche of different substrates. Even the SRB and TDLO form a stable sulfureta.

Though high numbers were not retrieved from ice in the present study, the anaerobes were more frequently encountered. Despite severe conditions wide range of micro-organisms have been identified or cultured from snow and ice in central Antarctica (Vincent, 1988). It has been reported that in thick ice the bacterial production exceeded primary production of micro algae (Grossmann and Diekmann 1994). Numbers upto 186 m^{-1} in the ice core at 0-20 cms have been reported by Gosnik *et al.* (1993). CFU and AODC values in ice were found to be 6-8.5 times higher than in the surrounding waters by 7 lanowski and Donachie(1993).

Bacterial abundance and the potential production depicted by their viability, point out to a productive system. Earlier studies from some of these lakes show very high $V_{\text{max}} \text{ cell}^{-1}$. This seems to be in agreement with the conclusion by Kogure *et al.*, (1986) who have shown that the abundance and production rate of bacterioplankton in Antarctica are as high as those in equatorial or temperate waters i.e. $0.45\text{-}5.2 \times 10 \text{ cells l}^{-1} \text{ d}^{-1}$ range. This has been stressed by pointing out that the harsh environment in Antarctica selected for robust and resilient flora (Vincent, 1988). The greater numbers of anaerobic forms and the higher propensity to grow under reduced conditions in fresh water samples could also indicate one such strategy to survive in low temperature and high oxygen concentrations. It is known that the reducing/anaerobic environment decreases the metabolic range and perhaps this contributes to their increased capacity to survive.

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