Truce with oxygen – Anaerobiosis outcompete aerobiosis in the Antarctic lacustrine bacteria

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The total number of bacteria counted directly by epifluorescent microscopy showed that they ranged from 10^8–10^9 l^{-1} in Antarctic lake water samples. The percentages of retrievable viable counts (RVC) of anaerobic bacteria (AnB) was greater than aerobic counts. Among the different groups of anaerobes, the order of retrieval was *Thiobacillus denitrificans* like organisms (TDLO) > fermentative bacteria (FB) > sulfate reducing bacteria (SRB). The total direct anaerobic viable counts (TDAnVC) was one order more than the total direct aerobic viable counts (TDAeVC). Laboratory experiments with one of the lake-isolates indicated that there was a tendency to express higher viability of 61% at redox potential (Eh) ranging from –281 to –335 mv. It is suggested that the disposition to express increased viability under reducing conditions is a strategy to counteract stress due to supersaturation of oxygen in the cold lacustrine environment.

STUDIES in the antarctic region have stressed on the biomass and activities in the terrestrial and aquatic ecosystems[1–3] and have pointed out that the bacteria-based food webs are as important in overall energy and material cycling in the high latitude oceans as they are at lower latitudes[4]. In the course of analysing antarctic water samples from lakes, the unusual phenomenon of retrievable viable counts (RVCs) of total anaerobic bacteria (AnB) far exceeding the aerobic ones in the form of colony forming units (CFUs) was noticed. The averages of general heterotrophic AnB were > *Thiobacillus denitrificans* like organisms (TDLO) were > lactate and acetate fermentors (FB) were > sulfate-reducing bacteria (SRB) were > aerobic bacteria (AB) in lacustrine environment where the dissolved oxygen is known to range from 10.4–13.8 mg l^{-1} (ref. 5). It has been a common observation that the retrievable aerobic counts in the form of CFUs are generally higher than anaerobic counts in surface layers of any water body. Exceptions are from specialized ecosystems like offshore oil wells[6] or deep, anaerobic, alkaline aquifiers[7], where the anaerobic counts are higher than the aerobic. In the antarctic lake waters where the oxygen concentration is generally high, it was intriguing to encounter more anaerobic bacteria than aerobic forms in the surface waters. The importance of the anaerobic forms *vis-à-vis* the aerobic ones have however received little attention. SRB have been recovered from anaerobic bottom waters by Konda *et al.*[8].

Further, it was observed that the direct viable counts carried out in these water samples showed higher viability under reducing conditions. Does the observation reflect the physiological adaptation in bacteria to extreme conditions? This paper will discuss these findings along with laboratory experiments to corroborate the observations.

During the 13th Antarctic expedition (Dec. 1993–March 1994), microbiological sampling was carried out from Antarctic lakes (including lake Priyadarshini) around the Maitri Station. Water samples were collected in sterile Erlenmeyer flasks and stored in ice until analyses at field station, within 5–6 h of collection.

For total direct counts (TDCs), an aliquot of sample was immediately preserved with 2% formalin. The fixed sample was stored in the cold and further processed at the National Institute of Oceanography (NIO), Goa. Bacteria were estimated using the acridine orange direct count (AODC) method as described by Hobbie *et al.*[9] and the counts are expressed as number per litre.

Total direct viable counts (TDVCs), were estimated as outlined by Kogure *et al.*[10,11] using a mixture of piromedic, pipemedic, nalidixic acid and yeast extract. To differentiate anaerobic viability from aerobic, a reductant i.e. Na_2S at a final concentration of 0.0125% (125 ppm) was added before incubating the samples. These experiments were carried out in screw-capped tubes filled to the brim to minimize oxidation. As the tubes were incubated at low ambient temperatures of 8–12°C, the period of incubation was extended to 12–16 h. The samples were fixed and the numbers were estimated as described above.

For retrievable counts (RC), CFUs were counted on suitable media by spread plating. Plating was carried out within a few hours of collection. Nutrient agar prepared with freshwater was used to estimate the CFU of AB and agar shake tubes for AnB. 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%, the heat shock to the microbes was minimal. In addition, other special media like modified Hatchikian's medium[12] and Leiske's medium[13] were used for enumerating specific anaerobic groups like SRB, FB and TDLO, respectively. All colonies that were not black by sulfide precipitation were counted as FB. The anaerobic CFU were AnB, FB, SRB, and TDLO. The plates were incubated at 8–10°C for 10–20 days and the tubes for ca 30 days. All samples have been analysed in replicates and only the average values of 20 samples from different lakes are presented.

A laboratory experiment was set up to find out whether the viability of bacteria is increased under

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Table 1. Aerobic versus anaerobic counts in the Antarctic lake water samples

	No. × 10^8 l^{-1} (epifluorescent counts)			No. × 10^4 l^{-1} (retrievable viable counts)				
Bacterial parameter	TDC	TDAeVC	TDAnVC	AB	AnB	FB	SRB	TDLO
Average (± SD) $n = 20$	20.45 (± 24)	1.57 (± 1.42)	18.12 (± 21.48)	0.11 (± 0.16)	16.23 (± 12.14)	1.19 (± 1.19)	0.27 (± 0.27)	5.08 (± 11.01)
Percentage of TDC		7.60	88.60	0.0001	0.01	0.001	0.0001	0.003

Microscopic counts – TDC, Total direct counts; TDAeVC, Total direct aerobic viable counts; and TDAnVC, Total direct anaerobic viable counts.

Retrievable plate/tube counts – AB, Aerobic bacteria; AnB, Anaerobic bacteria; FB, Fermentative bacteria; SRB, Sulfate-reducing bacteria; and TDLO, *Thiobacillus denitrificans*-like organisms.

reducing conditions at low temperatures. A gram negative psychrotrophic bacteria, 142A isolated from one of the lakes was chosen for this study as it could grow both at ambient and cold temperatures. The isolate was incubated at increasing concentrations of a reductant sulfide (63–188 ppm) at 5°C for 16 h and the viability estimated using Kogure's method[10,11]. Viability is expressed as percentage of total viable counts in the control observed after 16 h.

The TDCs in these oxygen supersaturated lakes were 20.45 × 10^8 cells l^{-1} whereas the percentage of direct viable aerobic and anaerobic counts were 7.6 and 88.6%, respectively. The retrievable anaerobic viable counts (RAnVCs) were 4 orders less than the TDC and 2 orders higher than the retrievable aerobic viable counts (RAeVCs). While the retrieval of total anaerobic counts was about 0.01%, that of TDLO and FB were 0.003 and 0.001 of TDC, respectively, i.e. ratio of distribution of AnB, TDLO, FB, SRB and AB in the total population was 100:30:10:10:1, respectively (Table 1).

The estimation of the total number of bacteria are in the same order 10^8 or 10^9, as described by Laybourn-Parry *et al.*[14] though they were 2 orders lower than that observed by Ramaiah[3]. Lower retrievability as compared to viability indicated that most of the cells were in viable but nonculturable state[15]. The high standard deviation encountered in the case of retrievable viable counts is indicative of the variability in twenty odd samples collected from different lakes (Table 1).

The solubility of oxygen in antarctic waters is high and is generally higher in the lakes than in sea water[16,17]. Ingole and Parulekar[5] reported that the dissolved oxygen in the fresh water lakes in Schirmacher oasis, East Antarctica, ranged from 10.4 to 13.8 mg l^{-1}. Dissolved oxygen in lake Priyadarshini alone, which is close to the Indian station, varied from 8.71 to 12.92 mg l^{-1} (ref. 18). Higher amounts of dissolved oxygen in these waters could be attributed to the higher solubility of the gas at lower temperatures.

Though oxygen is an effective electron acceptor, making energy conversion with high efficiency possible, it could be considered not so effective – or rather toxic

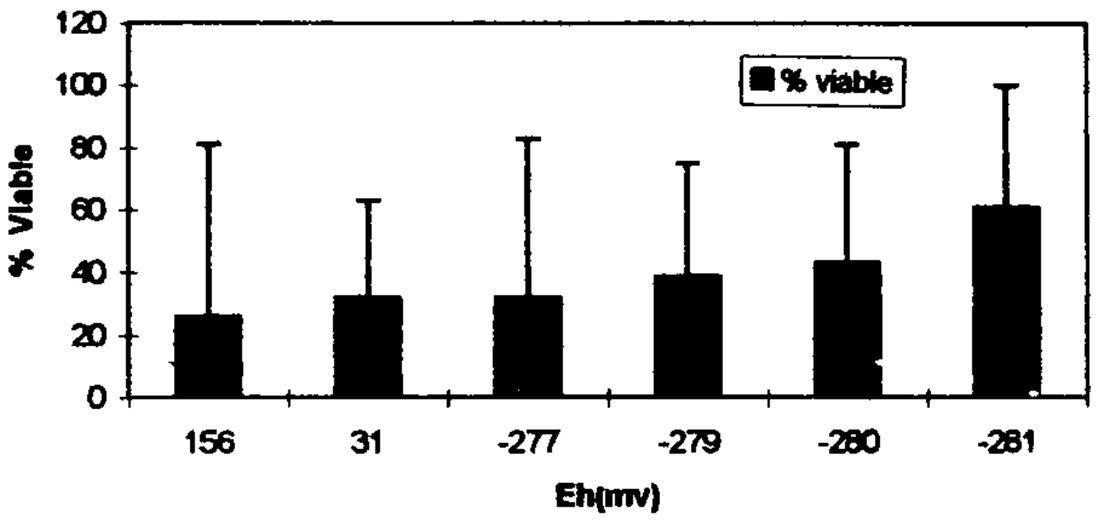

Figure 1. Bacterial viability at different Eh ($n = 10$ for oxidizing and 20 for reducing conditions).

when its concentration is high[19]. Even aerobic or facultative organisms live only in a balanced truce with oxygen[20]. Many strictly aerobic bacteria that form colonies from single cells on petridishes exposed to air can tolerate gas mixtures up to 40% oxygen by volume but fail to grow at 50%. Also 100% oxygen is usually considered to suppress growth. To many species of bacteria oxygen is inhibitory and causes a repellent response. Even obligate aerobes could be repelled by high concentrations of oxygen[21]. This is perhaps why the microorganisms have evolved a strategy to express increased viability only when the Eh is reduced. Perhaps they thrive better in anaerobic niches in highly aerobic water.

Experiments carried out on one of the isolates substantiate our field observations that viability is better expressed when conditions are reducing and temperature cold. The viability of cells incubated under aeration was only about 25% of the control when compared to 61% at 188 mv. Viability was maximum at 61% when the Eh ranged from –335 to –281 mv at 5°C (Figure 1). Only the Eh at the end of incubation is shown in the figure. Parallel experiments carried out at room temperature showed that solubility and therefore the availability of oxygen was lower. The expression of viability under anaerobic condition in these experiments was also lower (data not shown).

Thus, the field observations indicate that the potential expression of anaerobic growth by the bacterial population (viable and retrievable numbers) is much higher than the aerobic when the waters have generally high dissolved oxygen content. This paradoxical expression of increased anaerobic viability under oxygenated condition is more evident in the lacustrine environment than the marine. It is suggested that this phenomenon could be a strategy adopted by bacteria to express viability under reducing conditions when the concentration of dissolved oxygen in the surrounding waters is high/saturating. Further experiments are underway with microaerophilic, highly aerobic and strictly anaerobic isolates to understand the expression of viability in the highly oxidized environment.

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