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# Distribution of heterotrophic bacterioplankton in the Indian sector of Southern Ocean

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**Abstract**

Study was carried out on the distribution of bacterioplankton in the Indian sector of Southern Ocean. Total Heterotrophic Bacterial (THB) counts were maximum ($107x10^4$/CFU/ml) in water samples collected from 200 m depth and lowest ($15.1x10^3$/CFU/ml) at 3730 m. Of the 250 strains isolated from the water samples, 9.2% were gram positive and 4.8% gram negative. The important genera encountered were *Pseudomonas, Aeromonas, Vibrio, Acinetobacter, Micrococcus, Staphylococcus, Corynebacterium, Flavobacterium, Chromobacterium, Moraxella, Bacillus* and *Planococcus.* Most of the isolates (94%) were capable of lipase production followed by gelatinase (40%) and amylase (32%).

**Keywords:** Heterotrophic, bacterioplankton, enzymes, Indian sector, Southern Ocean

## Introduction

Major advances in our understanding of marine bacterial diversity have been gained through studies of bacterioplankton which are microscopic in size but often very abundant. Reports state that world ocean contains 3.1 x $10^{28}$ bacterial cells (Karner *et al.,* 2001). They are very important for cycling of carbon in the ocean and as a major component of food webs. The Southern Ocean is declared as new ocean in the year 2000 by International Hydrographic Organization (IHO), with a total area of 20.327 million sq km; the fourth largest ocean in the world (Russell, 2007). The Southern Ocean is very deep, with very limited areas of shallow water with an average depth of 4000 m. Southern Ocean has been characterized as high nutrient, low chlorophyll ocean (Chisholm and Morel, 1991). The low bacterial growth may be due to low dissolved organic matter and iron in the Southern Ocean (Church *et al*., 2000). The aim of the present paper is to study the quantitative and qualitative distribution of total heterotrophic bacteria at selected stations in the Southern Ocean and the hydrolytic enzyme production potential of the isolates.

## Material and Methods

***Sample Collection:*** Water samples for the present study were collected during cruise No. 200 in January - February, 2004, by the Oceanographic Research Vessel (ORV) *Sagar Kanya* of Dept. of Ocean Development Govt. of India. Twelve stations representing 16 various depths (200 to 4,780 m) were covered which extended between latitude 27° 14' 00'' S - 44° 59' 55" S and longitude 50° 32' 00" E - 57° 29'55"E (Table 1 & Fig. 1). Niskin sampling bottles of 2 litre capacity were used for sampling and the samples were immediately preserved at -20°C. The temperature, salinity and depth of sampling stations were recorded using the Sea-Bird CTD (conductivity-temperature-depth) onboard.

***Estimation of THB:*** Standard Plate Count method (spread plate) was adopted for estimating THB in the samples using ZoBell's 2216 e agar. The plates were incubated at 18 ± 2°C for 1 to 2 weeks.

Table 1. Details of sampling stations during *Sagar Kanya* cruise No. 200 in Southern Ocean

| Station No | Date | Latitude (S) | Longitude (E) | Sampling Depth (m) |
|---|---|---|---|---|
| 9 | 30.01.2004 | 31°00'39" | 44°59'40" | 2200 |
| 11 | 01.02.2004 | 32°50'30" | 44°54'06" | 1166 |
| 13 | 02.02.2004 | 35°04'46" | 44°59'46" | 2200 |
| 17a | 06.02.2004 | 40°57'25" | 44°59'52" | 3730 |
| 17b | 06.02.2004 | 40°57'25" | 44°59'52" | 3900 |
| 21 | 08.02.2004 | 43°01'11" | 45°00'27" | 2950 |
| 23 | 10.02.2004 | 45°01'23" | 45°06'08" | 1580 |
| 30 | 14.02.2004 | 51°59'51" | 44°59'56" | 3050 |
| 31 | 15.02.2004 | 53°00'00" | 44°59'45" | 300 |
| 32a | 15.02.2004 | 54°01'10" | 43°00'13" | 200 |
| 32b | 15.02.2004 | 54°01'10" | 43°00'13" | 3896 |
| 33a | 16.02.2004 | 54°59'50" | 45°00'14" | 200 |
| 33b | 16.02.2004 | 54°59'50" | 45°00'14" | 4137 |
| 33c | 16.02.2004 | 54°59'50" | 45°00'14" | 4780 |
| 34 | 17.02.2004 | 56°00'14" | 45°00'25" | 2200 |
| 38 | 24.02.2004 | 44°59'55" | 57°29'55" | 200 |

The colonies were counted and expressed as colony forming units per ml of the sample. Colonies were isolated, repeatedly streaked on nutrient agar plates for purity and preserved in nutrient agar vials overlaid with sterile liquid paraffin. The cultures were identified up to generic level.

***Hydrolytic enzyme production:*** Nutrient agar medium supplemented with starch (1%), gelatin (2%) and tributyrin (1%) were prepared separately. The cultures were spot inoculated and the plates were incubated for 3 to 5 days. Starch agar plates were flooded with Lugol's iodine solution and observed for the clearance zone around the colony for amylase production. The gelatin agar plates were flooded with 15% mercuric chloride and observed for the clearance zone around the colony for gelatinase production. For lipase, tributyrin agar plates were observed for clearance zone.

## Results

Total Heterotrophic Bacteria count ranged from $1.51x10^4$ to $107x10^4$ CFU/ml in the sampled stations (Fig. 1). THB was found to be the highest at station No. 33 and lowest at station No. 17. Surface water samples showed higher range of THB than deeper water.

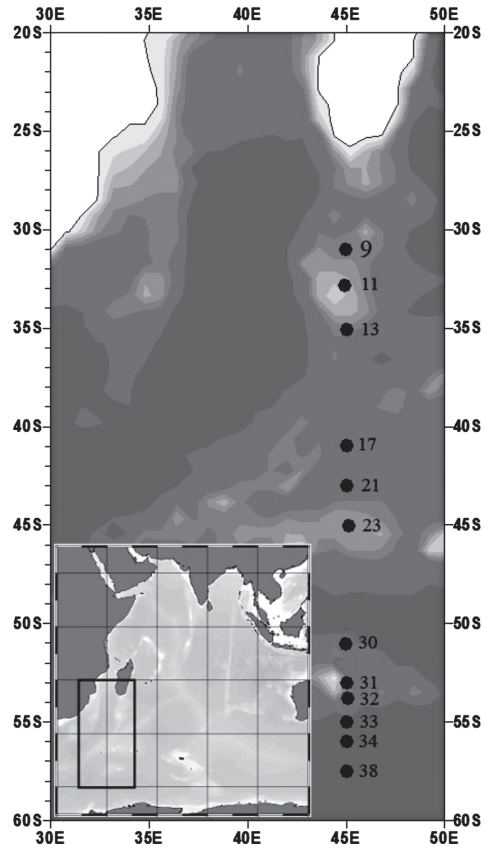


Fig. 1. Stations for bacterioplankton sampling in Southern Ocean

***Generic composition of heterotrophic bacteria:*** Of the 250 strains isolated from the water samples, 23 were positive rods, 69 positive cocci, 146 negative rods and 12 negative cocci. The important groups isolated were *Pseudomonas, Aeromonas, Vibrio, Acinetobacter, Micrococcus, Staphylococcus, Corynebacterium, Flavobacterium, Chromobacterium, Moraxella, Bacillus, Planococcus* and Enterobacteriaceae (Fig. 3). *Pseudomonas* was the dominant genus followed by *Vibrio, Acinetobacter, Micrococcus* and *Aeromonas*. Enterobacteriaceae, *Planococcus, Moraxella, Chromobacterium* and *Bacillus* were found to be rare in the water samples.

***Diversity indices:*** Similarity between stations was computed using PRIMER 5.2.2 software package for Windows (Fig. 4). Maximum similarity (96%) was noticed between station 9 (2200 m) and 33C (4780 m). About 50% overall similarity could

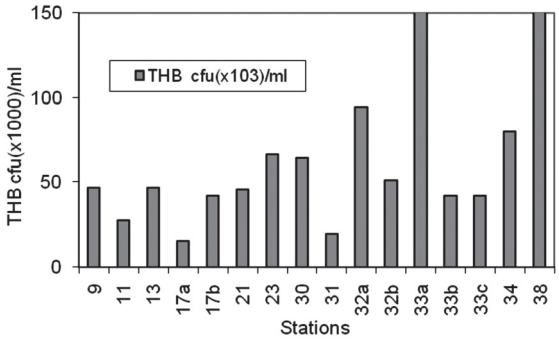


Fig. 2. Total Heterotrophic Bacterial (THB) population in the Southern Ocean

be observed between the stations studied. Stations 13, 32B, 31, 38, 11 and 34 formed a cluster with 57% similarity. Stations 23, 30, 21, 32A, 9 and 32C formed another cluster with 67% similarity whereas stations 17A, 33A and 33B formed a cluster with 58% similarity.

***Extracellular Enzyme Production:*** Ninety-four percentage of the strains showed ability to produce lipase, 40% produced gelatinase and 32% amylase. Of the strains 51% were able to produce only two types of extracellular enzymes. In the case of amylase production about 15% showed more than 2 cm clearing zone and for gelatinase and lipase 14% and 10% respectively.

Of the various genera capable of producing lipase, *Pseudomonas* (21%) was found to be the dominant genus followed by *Vibrio* (16%), *Aeromonas* (12%), *Acinetobacter* (12%), *Micrococcus* (12%), *Staphylococcus* (8%), *Moraxella* (5%), *Bacillus* (3%), *Flavobacterium* (5%), *Corynebacterium* (3%) and *Chromobacterium* (2%). *Vibrio* was found to be the dominant genus for gelatinase production followed by *Micrococcus* (15%), *Pseudomonas* (14%), *Acinetobacter* (13%), *Staphylococcus* (5%), *Moraxella* (5%), *Bacillus* (3%), *Flavobacterium* (3%) and *Corynebacterium* (3%). *Vibrio* constituted about 38% of the amylase producers followed by *Acinetobacter* (16%), *Pseudomonas* (11%), *Micrococcus* (9%), *Flavobacterium* (6%), *Chromobacterium* (4%), *Moraxella* (4%), *Aeromonas* (4%), *Staphylococcus* (3%) and *Bacillus* (1%). Vibrios were the most dominant genus capable of producing the various enzymes *i.e*., 97%, 79%, 73% for lipase, amylase and gelatinase, respectively. Lipase producing forms were very high among the genera whereas gelatinase and amylase producers were comparatively less (Fig. 5).

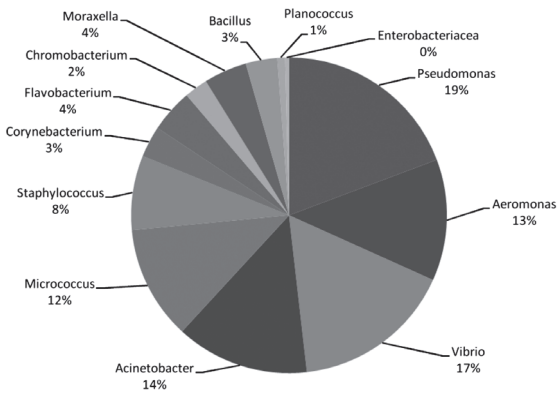


Fig. 3. Generic Composition of THB isolated from Southern Ocean

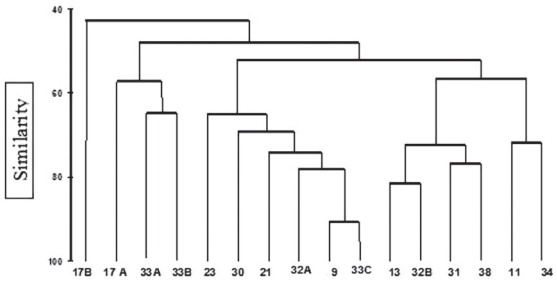


Fig. 4. Similarity between various stations from Southern Ocean

## Discussion

Marine bacterioplankton represent one of the most thoroughly studied environmental communities on the planet (Giovannoni and Stingl, 2005). In the present investigation, an attempt has been made to study the distribution of heterotrophic bacterial population from selected stations of Southern Ocean. Compared to Arabian Sea and Bay of Bengal, the Total Heterotrophic Bacteria (THB) of Southern Ocean harboured lower bacterial population (5.0 to 205.0 cells $ml^{-1}$) (Ramaiah *et al*., 1994., Church *et al*., 2000; Fernandes *et al*., 2008). Higher population of Arabian Sea ($0.52\pm0.29 \times 10^8$ cells $l^{-1}$) may be due to the upwelling and higher primary productivity. Southern Ocean is characterized as high nutrient, low chlorophyll ocean. The low bacterial growth may be

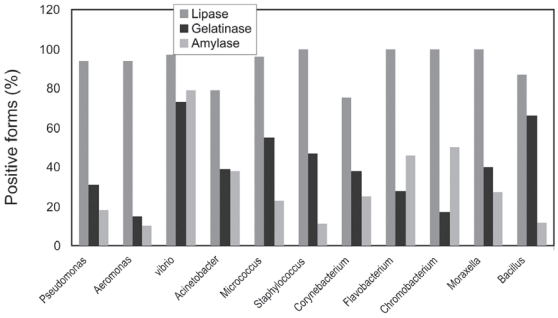


Fig. 5. Percentage of isolates capable of hydrolytic enzyme production

due to low dissolved organic matter and iron in the Southern Ocean (Pakulski *et al*., 1996, Tortell *et al*., 1996). Thus iron deficiency might restrict the growth of heterotrophic bacteria.

Surface water samples showed higher range of THB than deeper waters. Normally, the highest bacterial numbers are found in the productive euphotic zone. In deeper part of the Ocean almost all environmental factors are more or less same except pressure (Nybakken, 1982). The hydrostatic pressure rises by about 1 atm for every 10 m depth. The high-pressure in the deep sea affects the enzyme systems of bacteria. Mitskevich and Kriss (1975) has reported the peculiarities of quantitative distribution of heterotrophic bacteria at various depths in the water column of the western part of the Indian Ocean between 23° N and 44° S latitudes.

Only 32% of the strains in the samples collected during the present survey showed ability to produce extracellular amylase. Bacteria present in the surface water were more active in amylase production. In the case of lipase, 94% of strains produced extracellular lipases. *Pseudomonas* was found to be the dominant (21%) genus followed by *Vibrio* (16%). Ramaiah *et al*. (1994) reported the presence of *Pseudomonas, Vibrio, Chromobacterium, Aeromonas, Acinetobacter* and *Moraxella* in the Southern Ocean. These two genera were dominant in the surface water and therefore the surface water had more active producers of lipase. From the surface to deeper part of the ocean, the percentage of lipase producing forms decreased. Gelatinase producing forms were found to be maximum among the micro flora from deeper part of the Southern Ocean compared to those from surface water. Vibrios were found to be the dominant form in the case of gelatinase production. Therefore this phenomenon may be due to differential distribution of vibrios at various depths. Vibrios were found to occur at higher percentage in deeper parts of the ocean. However, Davey *et al*., (2001) noted that high Cytophaga-like bacterial abundance and proteolytic activity co-occurred in surface waters of the northeast Atlantic Ocean.

Boetius and Lochte (1996) found that deep sea bacteria from Arctic sediments have high proteolytic activity exceeding that of the shelf and upper slope bacterial assemblages by an order of magnitude. Enrichment experiments proved that the production of extracellular enzyme was adopted based on the availability of utilizable compounds. The isolates were found to be versatile in enzyme production contributing to the biogeochemical cycles in the region. Works on sediment microflora would be highly promising in the furtherance of the understanding on the ecology of this region.

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