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Bacterial Flora in Marine Sediments of the Eastern Antarctic Region with Special Reference to Berkilen Bay

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In microbial analysis of marine muddy sediments from the Berkilen Bay area near Jinnah Antarctic Base of Pakistan, bacterial counts of aerobic heterotrophs were determined on Tryptic Soya Agar with additional NaCl (3.5%) incubated at 0°, 5°, 10°, 25° and 37° C for 2 days to 4 weeks. The mean bacterial count (Colony Forming Units, CFU/g) of sediment was $1.2x10^4$ $CFUg^{-1}$ at 0°C; $2.6x10^3$ $CFUg^{-1}$ at 5°C; $1.1x10^4$ $CFUg^{-1}$ at 10°C; $2.7x10^4$ $CFUg^{-1}$ at 25°C and $2.3x10^4$ $CFUg^{-1}$ at 37°C. Bacteria isolated were psychrotrophs rather than psychrophiles. Among the identified genera gram positive dominated the gram negative. Genera isolated in the order of predominance were *Micrococcus, Moraxella, Staphylococcus, Bacillus, Pseudomonas* and *Flavobacterium.* The percentage of unidentified bacteria was quite low.

Key words: Marine sediments, Bacterial flora, Antarctic region, Berkilen Bay.

Introduction

Most of the biosphere is cold, 14% of the lithosphere's surface being in polar region and approximately 90% of the hydrosphere being 0°C or cold (Morita 1993). The Antarctic region constitutes an integral portion of this psychrosphere, representing a very unique ecosystem.

Though the Antarctic environment is extreme for living conditions, its lakes and ponds provide favorable habitat for microorganisms (Nagashami *et al* 1990). Also coastal terrestrial environment of the Antarctic continent shows strong relief in very variable harsh environmental conditions and short periods of favourable climate for biological activity (Bolter 1992). Such variability results in growth of diverse group of microorganisms (Sieberth 1967; Nedwell and Floodgate 1971).

Considerable microbial research has been undertaken in the extreme habitat of Antarctica. The present paper reports the results obtained from microbial analysis of marine sediments collected from Berkilen Bay during the Pakistani Scientific Expedition (1992-93). Although the main objective of the present study was to identify the native bacterial flora of the region at different temperatures, it also provides baseline data for assessment of other microbiological aspects such as role of these bacteria in sustenance of ecosystem.

Materials and Methods

A team of marine scientists of National Institute of Oceanography (NIO), Pakistan carried out sea bed sediment sampling on board M/V Mangen during the 2nd Pakistan Antarctic Expedition in the Brekilen Bay, Antarctica (Fig 1). A total of 20 sampling stations were located at approximately 1 km interval. A ven veen type of grab sampler was used for sediment sampling. Some sediment samples were provided to the microbiologist for the laboratory studies. The samples were stored at - 4 °C till processed. The data of only 10 sampling stations is presented in this paper. The geographic locations and collection date of samples are listed in the Table 1.

Cultural method. Media. Different media were tested for isolation of organisms coming from such an extreme environment. On the basis of preliminary results, Tryptic Soya Agar (TSA) (Oxoid) supplemented with a salt (NaCl) concentration of 3.5% was selected (Ramsey and Stannard 1986). This salt concentration was chosen because it is near optimal for the growth of organisms from marine environment.

Bacteriological analysis. Colony forming units (CFU) of aerobic heterotrophs were determined using surface spread plate method by placing 0.1 ml of appropriate dilutions of sediment samples on TSA (with 3.5% NaCl) in five sets. Each set was incubated at five different temperatures (0°, 5°, 10°, 25° and 37° C) to assess the temperature requirement of bacteria. Colonial counts were determined after 48 hours to four weeks depending on the temperature of incubation.

Isolation and identification of organisms. Three representative bacterial colonies of each of the predominant types from each plate were picked and identified according to the scheme 1 (Zuberi *et al* 1987). After checking for purity, the

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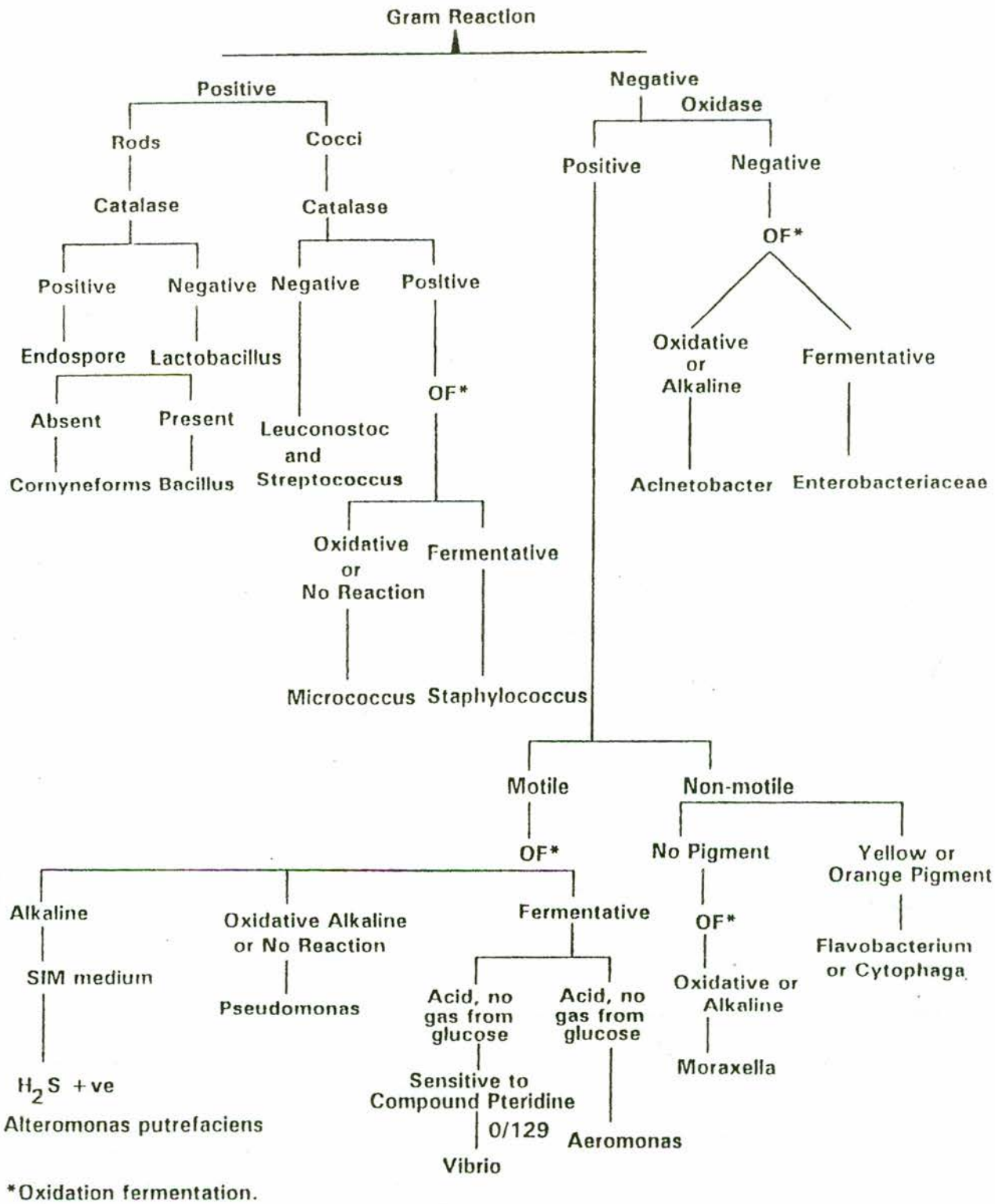

Scheme 1. Isolation and identification of bacteria

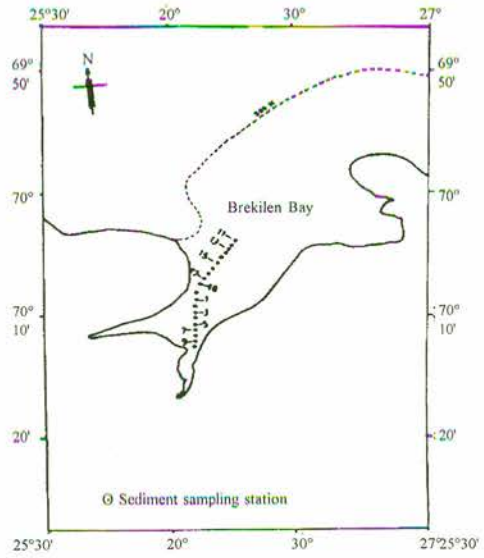

Fig. 1 Sampling area of the 2nd Pakistan Antarctic Expedition.

Table 1
Geographical characteristics and dates of collection of samples

No.of samples	Code of samples	Geographical locations Latitude	Longitude	Date of collection
1	1	70 08.02S	26 00.30 E	11-2-93
2	3	70 09.40 S	26 00.20 E	11-2-93
3	5	70 10.55 S	25 59.52 E	11-2-93
4	7	70 11.50 S	25 58.00 E	11-2-93
5	9	70 12.60 S	25 57.00 E	11-2-93
6	11	70 03.04 S	26 16.15 E	14-2-93
7	13	70 04.07 S	26 13.68 E	14-2-93
8	15	70 05.02 S	26 10.37 E	14-2-93
9	17	70 06.02 S	26 07.91 E	14-2-93
10	18	70 06.62 S	26 06.90 E	14-2-93

Table 2
Bacterial count of sediment samples at different temperatures

Sediment samples	Temperature of Incubation				
	0°C	5°C	10°C	25°C	37°C
01	Nil	Nil	Nil	$3.2x10^3$	$4.0x10^2$
03	$3.7x10^3$	$6.0x10^2$	$1.4x10^3$	$6.0x10^2$	$1.9x10^3$
05	Nil	$2.3x10^3$	$2.1x10^3$	$5.0x10^2$	$6.0x10^4$
07	$8.0x10^3$	$1.2x10^4$	$7.2x10^4$	$1.5x10^5$	$1.0x10^5$
09	$1.0x10^6$	$1.3x10^3$	$7.5x10^3$	$4.2x10^3$	$1.2x10^4$
11	Nil	$5.4x10^3$	$2.0x10^2$	$6.2x10^3$	$3.7x10^3$
13	$5.0x10^3$	$2.6x10^3$	$3.0x10^3$	$8.0x10^4$	$1.0x10^4$
15	$3.0x10^3$	$2.0x10^3$	$6.4x10^3$	$1.6x10^3$	$3.6x10^4$
17	Nil	Nil	$2.8x10^3$	$9.0x10^3$	$2.0x10^3$
18	$2.0x10^2$	Nil	$7.5x10^3$	$1.5x10^4$	Nil

isolated strains were tested for forms, gram reaction, motility, oxidase, catalase and oxidation/fermentation (O/F). Isolated organisms were subcultured on TSA slants (with 3.5%NaC1) and stored at low temperatures (0°C to 10°C).

Results and Discussion

Bacterial counts are presented in Table 2. Mean bacterial count obtained at 0°C was $1.2x10^4$ CFU g^{-1} with a range of $2.0x10^2$-$1.0x10^5$ CFU g^{-1}. Out of ten samples four samples (no. 1,5,11 and 17) did not show any growth at this temperature, though the growth was obtained at higher temperatures. For these samples probably incubation time and temperature were conducive, since both these factors influence the bacterial growth. At low temperatures the rate of growth slows down and more time is required. It is also likely that the culture medium was less suitable for the organisms. In an enriched medium like TSA, the maximum temperature for growth of organisms might have been greater than 21°C; in other words, nutritional status of the medium influences the maximal temperature for growth of a microbe (Ferroni and Kaminski 1980).

At 5°C, mean bacterial count was $2.5x10^3$ CFU g^{-1} with a range of $6.0x10^2$-$1.2x10^4$ CFU g^{-1}. Again three samples (sample no.1, 17 and 18) did not show any growth at this temperature. At 10°C mean bacterial count was $1.1x10^4$ CFU g^1 with a range of $2.0x10^2$-$7.2x10^4$ CFU g^{-1}. All the samples except no.1 showed growth. The count obtained, is consistent with the studies of line 1988. At 25°C all the samples showed growth and mean bacterial count was $2.7x10^4$ CFU g-1 with a range of $5.0x10^2$-$1.5x10^5$ CFU g^{-1}. This temperature appeared most suitable for organism of Brekilen Bay. Incubation temperature of 37°C seems to be unsuitable for the growth of organisms of such a cold environment. A mean bacterial count of $2.3x10^4$ CFU g^{-1} was however obtained in a broad range of $4.0x10^2$-$1.0x10^5$ CFU g^{-1} in nine of the ten samples indicating the presence of mesophilic bacteria. Fifty percent of the samples showed growth at all the temperatures, suggesting presence of diverse temperature requirements of organisms. Boyd and Boyd (1963) suggested that diversity of microbial population is related to the variability of environment, the main variable factor being the temperature of any environment.

Attempts were made to correlate the bacterial count with temperature, but no noticeable variance with respect to temperature was observed. Trend of bacterial counts was similar at all the temperatures except 5°C, where slightly lower counts were obtained. Similar observations were reported by Inove (1976) who observed no change in microbial count in direct proportion to the change in temperature. Therefore, it seems that temperature does not affect the change in microbial count especially when temperature is high enough

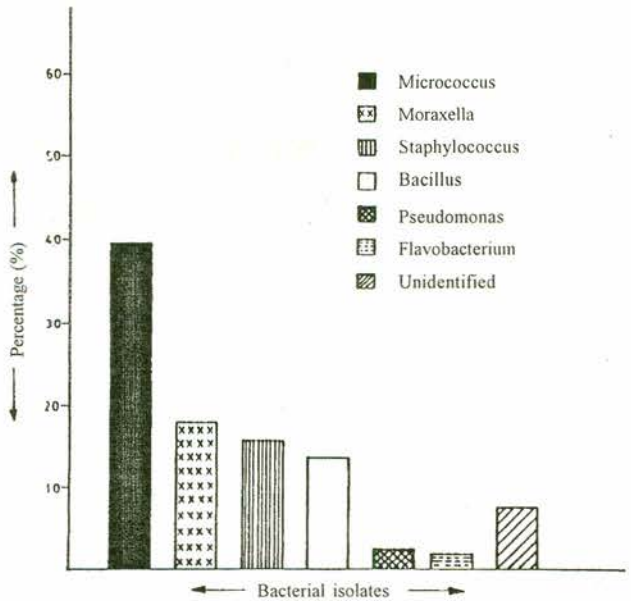

Fig. 2 Percentage distribution of bacteria genera isolated.

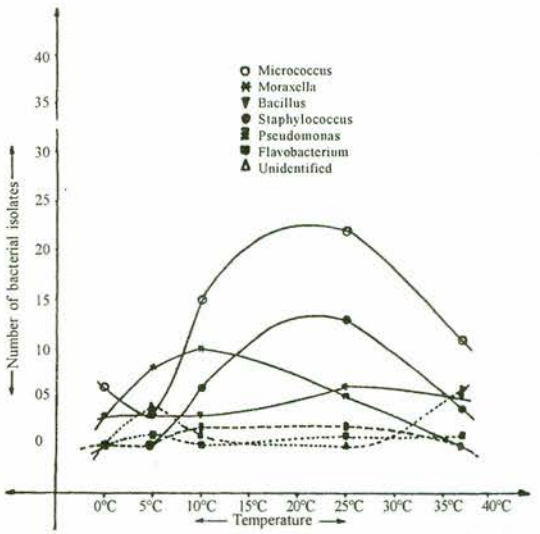

Fig. 3 Distribution of bacterial flora at different temperature.

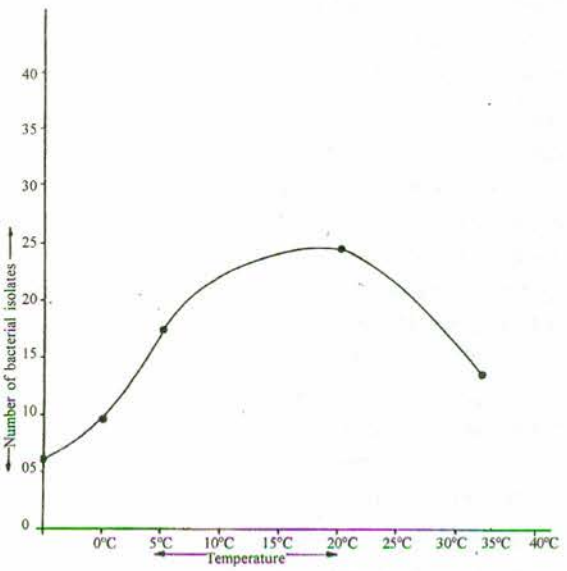

Fig. 4 Relation between temperature and number of bacterial isolate

for microbial growth. The low bacterial count obtained in the present study is contrary to those of the previous studies (Ramsay 1983; Sullivan and Palmisano 1984; Gibson *et al* 1990). However previous studies employed direct microscopy technique for the enumeration of bacteria. This may explain the large discrepancy between the results and confirms the influence of methodology (French and Smith 1986; Ramsay and Stannard 1986). Difference in sampling site and the nature of sample could also account for this discrepancy. The abundance of bacteria in the soil of Antarctica has been shown to be dependent on the local climatic conditions and the nutrient supply (Baily and Wynn-Williams 1982). High bacterial counts have been reported in soils from the penguin rockery (orthinogenic soils) compared to soils from the lakes (Shivaji *et al* 1989a). Low microbial population (10^4-10^6 cells g^{-1} dry soil) has been associated with pristine soils lacking in identifiable primary producers whereas high microbial population (10^6-10^9 cells g^{-1} dry soil) with soils contaminated by man or by birds (Line 1988).

During the course of study a total of 144 bacterial strains were isolated and identified upto generic level. Distribution of genera isolated at different temperatures is reported in Table 2. Diversity of microflora is apparent with varying temperatures. A shift from Gram negative to Gram positive organisms was observed in bacterial population with increasing temperatures (Ledduce and Ferroni 1979). Percentage of bacterial genera isolated in order of predominance, as reported in Fig 2, is in agreement with the previous taxonomic studies on the Antarctic soil microflora (Boyd and Boyd 1963).

Any attempt to delineate the dominant microflora of a region is limited by the number of samples and by the apparent diversity of microflora frequently occurring in close proximity to each other. The collective assessment of representative of the dominant flora of a range of samples as undertaken in the present study is not an entirely satisfactory compromise. However, it would appear that Gram positive organisms are abundant in the area of Brekilen Bay. This dominance of Gram positive organisms is consistent with the previous studies of Antarctic region (Johnson *et al* 1978; Ramsay 1983; Ramsay and Stannard 1986).

Microbial studies of specific regions of Antarctic Continental Shelf (Victoria dry valleys and McMurdo station area) have

also revealed the incidence of Gram positive bacteria (Madden *et al* 1979; Miller and Leschine 1984; Siebert and Hirsch 1988). The dominance of Gram positive organisms over other groups suggests their indigenous origin in the Antarctic environment (Line 1988), which is also reflected in the present study (Fig 2). Siebert and Hirsch (1988) inferred an ecophysiological observation that these organisms are well adapted to the local Antarctic conditions, a fact that excludes the possibility of their origin by human contamination.

Among the Gram positive organisms. *Micrococcus* was predominant at 0°, 10°, 25° and 37°C temperatures (Fig 3). The high incidence of *Micrococcus* is in accordance with the previous studies (Cameron *et al* 1970; Madden *et al* 1979 Johnson *et al* 1978; Johnson and Bellinoff 1981; Miller and Leschine 1984; Siebert and Hirsch 1988; Shivajii *et al* 1988). High incidence of Micrococcus at 25°C (Fig 3) reflects the psychrotrophic nature of *Micrococcus.* Isolation of psychrotrophic *Micrococcus* from Antarctic region has been reported by Siebert and Hirsch (1988). Some mesophilic *Micrococcus* were also isolated in the present study.

Bacilli have been reported to be rare in Antarctic soils uncontaminated either by man or birds (Boyd *et al* 1970). However these organisms were isolated at all the temperatures (Fig 3) in the present study, which reflects the transient allochthonous population of the area. Among the Gram negative organisms frequent isolation of *Morexella* at low temperature (Fig 3) indicates that it can survive fairly low temperatures (Zuberi *et al* 1986). Similar findings of the previous taxonomic studies also suggest an indigenous origin of *Moraxella* in this cold environment (Line 1988). Low incidence of Pseudomonas (Fig 3) is in agreement with the findings of Johnson *et al* (1978) and Siebert and Hirsch (1988).

In the present study maximum number of bacteria were isolated at 25°C, revealing their psychrotrophic nature. Figure 4 represents the correlation between number of isolates with the incubation temperature. The number increased with increasing temperature, maximum at 25°C and declined afterwards. Thus it can be concluded that majority of Antarctic heterotrophs are psychrotrophs rather than psychrophiles as has been proposed earlier (Delille and Perret 1989; Upton and Nedwell 1989).

Present findings contradict the report of Norkrans and Stehn (1978) who isolated zymogenous psychrophiles from the Arctic marine sediments. However psychrophiles tend to be isolated more frequently from thermally stable cold environments (i.e.<5°C) whereas psychrotrophs are more characteristic of thermally unstable cold habitats (Baross and Morita 1978). It should also be noted that relatively few truly psychrophilic species have been isolated even from permanently cold environments (Delille and Perret 1989; Upton and Nedwell 1989). Delille (1992) also reported that large majority of the bacteria isolated from sea water must be considered as psychrotrophs.

The predominance of psychrotrophic bacteria reflects the adaptation of these microorganisms to the cold environment (Shivaji *et al* 1989b). The local origin of the bacterial isolates can have a major importance in their physiological adaptation to extreme environment. In general, the results reported here are complementary to previous studies on microbial aspects of Antarctica. It can be concluded that Antarctic environment harbours a large diverse and active population of bacteria with dominating Gram positive organisms, majority of which are psychrotrophic in nature. Diversity of microflora is apparent with change of temperature in sediments of Berkilen Bay.

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