Proceedings of the

FIRST WORKSHOP ON SCIENTIFIC RESULTS OF FORV *SAGAR SAMPADA*

5-7 June, 1989, Cochin

Sponsored by

DEPARTMENT OF OCEAN DEVELOPMENT
&
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
NEW DELHI

Organized by

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
&
CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY
COCHIN

OCTOBER, 1990

Published by

Dr. P.S.B.R. JAMES
DIRECTOR
Central Marine Fisheries Research Institute
COCHIN - 682 031

Edited by

Dr. K.J. MATHEW
Central Marine Fisheries Research Institute
COCHIN - 682 031

Proc. First Workshop Scient. Resul. FORV Sagar Sampada, 5-7 *June*, 1989: 29 -32 (1990)

DISTRIBUTION AND BIOCHEMICAL ACTIVITY OF HETEROTROPHIC BACTERIAL POPULATION OFF MADRAS COAST

V. CHANDRIKA AND K. G. GIRIJAVALLABHAN*

Central Marine Fisheries Research Institute , Cochin- 682 031

ABSTRACT

The quantitative distribution and biochemical avtivity of heterotrophic bacteria capable of multiplying in organic media of Proteinaceous composition from, shelf and slope waters off Madras coast between 11° 30' N and 14° 30' N were studied during March, 1986. The percentage of samples yielding more than 100×10^5 / ml colonies was only 12 in number in depth range 0-10 metres. Pigmented bacteria was encountered more in this depth range. Faecal coliform bacteria was not encountered throughout the observations. Total bacterial count varied from 8.6×10^5 to 3.08×10^7 / ml in spread plate method of isolation. *Pseudomonas, Vibrio, Alcaligenes, Flavobacterium, Cytophaga, Agarobacterium* and *Chromobacterium* formed the predominant flora isolated. Their correlation with environmental parameters like temperature, salinity, oxygen and pH are discussed. The cultures were more proteolytic than amylolytic which indicated the process of organic decomposition and vigorous liberation of plant nutrients in these latitudes.

INTRODUCTION

Marine heterotrophic bacteria play a major role in the decomposition of dissolved organic substances. Their activities and relative abundance reflect the hydrological structure and nutrient levels in the marine environment (Oppenheimer, 1963). Also they form an important constituent of any ecosystem and can be considered as an index for monitoring environmental stress and changes. This paper reports the nature and distribution of the heterotrophic bacteria off Madras coast between 11 ° 30' N and 14° 30' N and 79° 54' E and 80° 24' E in relation to some environmental parameters such as temperature, salinity, dissolved oxygen and pH from the samples collected during Cruise 13A of FORV *Sagar Sampada* during 21st to 24th March, 1986.

MATERIALS AND METHODS

The quantitative and qualitative distribution of heterotrophic bacteria was assessed from water samples collected aseptically in precombusted water bottles from various depths (0 m, 10 m, 20 m, 30 m, 50 m and 75 m) in the shelf waters from six fixed stations (stations 473, 474, 475, 476, 477 and 478) from 21st to 23rd March, 1986 where the depth ranged from 0-91 m. The mid- depth samples from stations 476 and 478 were omitted since these 2 stations were relatively shallow. A rosette sampler was used for collecting water samples. Platings for bacterial enumeration were done within one hour of collection.

The temperature and pH of water samples were recorded and salinity and dissolved oxygen were determined by titration method using standard procedures.

The spread plate method was used throughout since this method has the advantages of a shorter incubation time, higher counts and more uniform colonial size over the streak plate on pour plate method (Buck and Cleverdon, 1961). Aerobic bacterial counts were obtained by plating 0.1 ml of an appropriate sea water dilution of the original sample on sea water Agar medium (10 g Peptone (Difco), 0.1 g Yeast extract (Difco) and 20 g purified Agar in 1000 ml aged sea water). The agar plates were kept in sterilised chamber for 1 hr to dry the surface prior to smear the 0.1 ml water samples over the surface. The plates inoculated were incubated at 20° C for 2 weeks and then colonies formed on the plates were counted according to standard methods. Faecal coliforms (*E. coli*) counts were made by using selective media Tergitol 7 Agar medium by the spread plate method.

Single colonies were picked up at random from each standard plate with 30 - 300 colonies and re-streaked on appropriate agar plates three times before a pure culture was established in agar slants. Standard bacteriological procedures were carried out to determine the bacterial genera and their identifications were accomplished according to Bergy's manual of Determinative Bacteriology by Breed *et al.*

Present address : * Madras Research Centre of CMFRI, Madras.

(1974) and other references (Zobell and Upham, 1944; Simidu and Aiso, 1962; Hayes, 1963; Shewan, 1963; Baumann *et al* ., 1972).

Results

Horizontal distribution

The heterotrophic populations in surface water were highest in 5 stations studied and total bacterial count varied from $8.6x10^5$ cells ml^{-1} to $3.08x10^7$cells ml^{-1}. The highest counts being usually obtained along the thermocline at 20 to 30 m. The percentage of samples yielding more than 100×10^5/ml colonies was only 12 in numbers in the depth-range of 0-10 m. Pigmented bacteria was encountered more in 0-10 metre depth range. Surface water temperatures at all stations were found to be rather uniform.

Vertical distribution

The temperature, dissolved oxygen and pH decreased while salinity increased with depth in all stations during the observation. Although stratification of the hydrological structure was observed, no definite stratification on the vertical distribution of heterotrophic bacteria was detected. The highest counts were obtained in surface samples in most of the stations except Station 474 where mid-depth samples contained more bacteria than in the surface samples.

The surface water temperature ranged from 28.1 to 28.9°C. The waters below the surface upto 30m showed a gradual decrease and ranged from 27.4 to 27.9°C. The temperature at 50m depth ranged from 24.5 to 27.7°C.

The dissolved oxygen content from surface to about 30 m depth showed uniform values around 4.8 ml/1.

The salinity of the surface waters ranged from 28.5 to 34.9‰. At 50 metres the salinity ranged from 29.4 to 34.8‰.

Determination of bacterial genera

A total number of 46 strains were isolated from all the samples and the strains were assigned to 7 genera including 12 strains of *Pseudomonas*, 10 strains of *Vibrio*, 5 strains of *Alcaligenes*, 7 strains of *Flavobacterium*, 8 strains of *Cytophaga*, 3 strains of *Agarobacterium* and one strain of *Chromobacterium* (Table 1).

A large number of yeasts were also present in the Tergitol 7 Agar plates after 24 - 48 hrs incubation but faecal coliforms (*E. coli)* was not encountered throughout the observation in this selective medium. All the 15 pigmented forms like *Flavobacterium* and *Cytophage* were encountered in the depth range 0-10 metres. An antibiotic producing purple-pigmented *Chromobacterium* was isolated from the sea water sample by using non selective isolation techniques. The culture produces 3 bromine containing metabolites. Anderson *et al.* (1974) showed that these compounds can inhibit the growth of selected human pathogens as well as certain marine bacteria. The organism is maintained in the laboratory for further studies of antibiotic properties.

Table 1. *Number and percentage of identified strains of marine bacteria isolated from off Madras coast*

Bacterial strain	No. of strains	Percentage
Pseudomonas	12	26.0
Vibrio	10	21.7
Alcligenes	5	10.8
Flavobacterium	7	15.2
Cytophaga	8	7.3
Agarobacterium	3	6.5
Chromobacterium	1	2.1

Discussion

The water collected at various depths gave a picture not only of the quantitative distribution of the bacterial flora but also generic composition (Table 1) and biochemical activity (Table 2). The flora consisted of physiologically active organisms exhibiting proteolytic (78. 3%) amylolytic and lipolytic activities. The present pattern of horizontal distribution of bacteria is comparable with those observed in Palk Bay in India (Velankar, 1955), Kamogaway in Japan (Simidu and Aiso, 1962) and Narrangansett Bay in Rhode Island, U.S.A. (Sieburth, 1967).

The actinomycetes and yeasts are known to play important role in degradation. In the present study although no specific attempt was made to enumerate these groups a large number of yeasts were isolated in Tergitol 7 Agar, reflecting their predominance in this area.

Like in other marine environments (Simidu and Aiso, 1962; Liston Colwell, 1963; Shewan, 1963; Simidu *et al.*, 1971; Baumamn *et al.*, 1972) same types of micro-organisms belonging to the genera *Pseudomonas, Vbrio, Alcaligenes and Flavobacterium* were isolated in Madras coast also. The numbers of each

of the taxonomically distinct groups showed the presence of variety of enzymes implicated in degradative processes.

TABLE 2. *Bio-chemical characteristics of 46 marine bacterial strains off Madras coast*

Characteristics	Frequency of occurrence(%)
1. Gram negative	98.0
2. Motility	47.6
3. Pigmented	32.6
4. O/F Dextrose fermentation	
Oxidative	36.8
Fermentative	30.0
No reaction	19.5
5. Proteolytic	78.3
6. Amylolytic	44.5
7. Lipolytic	20.6
8. Nitrate reducers	90.6
9. H_2S producers	12.5
10. Indole producers	—
11. Catalase producers	100.0
12. Sugar fermentation	
Glucose	40.6
Sucrose	35.8
Lactose	Nil
Maltose	55.0
Mannitol	42.6
13. Sensitivity towards Penicillin	
2.5 I.V./Disc.	12.6

Regarding vertical distribution, slight stratification of temperature, salinity, dissolved oxygen and pH is observed in this region. However, no stratification of heterotrophic aerobic bacteria is detected. Due to the relative shallowness of the stations, the mixing effect of water layers is quite evident as indicated by the slight stratification (Table 3) of hydrological parameters and the similar number of bacteria present in different depths. Total counts of bacteria off Madras region are correlated with environmental parameters and none of the correlations are found significant except depth versus temperature (Table 3).

The effect of temperature on micro-organisms is well documented (Ingraham, 1962; Rose, 1967). Most of the marine bacteria in temperate regions grow well at temperatures between 22 and 30°C. It was concluded that the maximum growth temperature for bacteria taken from tropic and temperate waters is only a few degrees above their environmental temperature (Zo Bell, 1962). The temperature never exceeded 28.5°C during the present observation providing sub-optimal temperature for the growth of marine bacteria. Thus correlation analysis in the present investigation indicated that temperature and other parameters have little effect on both horizontal and vertical distribution of heterotrophic aerobic bacteria off Madras region.

The effect of salinity and pH are probably more serious on the bacteria. It is well documented that faecal coliforms and non-marine bacteria die rapidly in water with high salinities and alkaline pH which may be the reason for not encountering faecal coliforms throughout the observation. Since the mortality of coliform and non-marine bacteria is high in sea water (Ketchum *et al.*, 1952; Carlucci and Pra-

TABLE 3. *Mean and std. deviation of different characteristics*

Characteristics	Mean	s.d.	Correlation matrix					
			1	2	3	4	5	6
1. Depth	17.200	14.583	1.000	0.172	0.105	-.266	-.834	-.377
2. pH	8.214	0.006	0.172	1.000	0.170	0.213	-.029	-.124
3. Salinity	31.694	2.326	0.105	0.170	1.000	-.008	0.217	-.085
4. Oxygen	4.814	0.414	-.266	0.213	-.008	1.000	0.226	-.163
5. Temperature	27.823	0.922	-.834	-.029	0.217	0.226	1.000	0.337
6. Total plate count	109.920	45.590	-.377	-.124	-.085	-.163	0.337	1.000

mer, 1969; Carlucci *et al.*, 1961; Jones, 1963) they do not constitute any significant portion of the heterotrophic bacterial population in this marine environment.

Acknowledgement

Authors are grateful to Dr. P.S.B.R. James, Director, Central Marine Fisheries Research Institute, Cochin for facilities extended to conduct this study and present this paper at the Workshop.

References

Anderson, R.J., M.S. Wolfe and D.J. Faulkner 1974. Autotoxic, antibiotic production by a marine Chromobacterium. *Marine Biology*, **27**: 281-285.

Baumann, L., P. Baumann, M. Mandel and R.D. Allen 1972. Taxonomy of aerobic marine bacteria. *J. Bacterial.*, **110**: 402-429.

Breed, R.S., E.G.D. Murray and N.R. Smith 1974. *Bergy's Manual of Determinative Bacteriology*. William - Wilkin's Col (Eds.), Baltimore, Md. U.S.A., 1994pp.

Buck, J.D. and R.G. Cleverdon 1961. The effect of Tweeb 80 on the enumeration of marine bacteria by the spread and pour plate method. *Limnol. Oceanogr.*, **6**: 42-44.

Carlucci, A.P, R.V. Scarpino and D. Pramer 1961. Evaluation of factors affecting survival of *Escherichia coli* in sea water. *Appl. Microbiol.*, **9**: 400-404.

Carlucci, A.P. and D. Pramer 1969. An evaluation of factors affecting the survival of *Escherichia coli* in sea water. II. Salinity, pH and nutrients. *Appl. Microbiol.*, **8**: 247-250.

Hayes, P.R. 1963. Studies on marine flavobacteria. *J. Gen. Microbiol.*, **30**: 1-9.

Jones, G.E. 1963. Suppression of bacterial growth by sea water. In : *Symposium on Marine Microbiologiy*, C. H. Oppenheimer (Ed.), pp. 572-579. Charles C. Thomas Publ., Springfield Ill.

Ketchum , B. H., J.C. Ayers and R.F. Vaccaro 1952. Process contributing to the decrease of bacteria in a tidal estuary. *Ecology*, **33**: 247-252.

Liston, J. and R.R. Colwell 1963. Host and habitat relationships of marine commercial bacteria, In : *Symposium on Marine Microbiology*, pp. 611-624 , Charles C. Thomas Publ., Springfield Ill.

Oppenheimer, C.H. 1963. *Symposium on Marine Microbiology*. Charles, C. Thomas Publ., Spring., U.S.A., 769. pp.

Rose, A.H. 1967. *Thermobiology*. Acac. Press, N.Y., U.S.A., 286 pp.

Shewan, J.M. 1963. The differentiation of certain genera of Gram-negative bacteria frequently eccountered in marine environments, C. H. Oppenheimer (Ed.), *Symposium on Marine Microbiology* pp. 499-521.

Seiburth, J.M. 1967. Seasonal selection of estuarine bacteria by water temperature. *J. Exp. Mar. Ecology*, **1**: 98-121.

Simidu, V. and K. Aiso 1962. Occurrence and distribution of heterotrophic bacteria in sea water, in Kamogawa Bay. *Bull. Jap. Soc. Sci. Fish.*, **28**: 1133-1141.

Velankar, N. K. 1955. Bacteria in the inshore environment at Mandapam. *Indian J. Fish.*, **2**: 96-112.

Zo Bell, C.E. 1962. Importance of micro-organisms in the sea. *Microbiol. Symp. Camden, New Jersey*, pp.107-132, Campbell Soup Co Camden, N.J., USA.