Available online at www.scholarsresearchlibrary.com

Scholars research library

Archives of Applied Science Research, 2011, 3 (5):593-598
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Prevalence and distribution of total heterotrophic bacteria from Kottaipattinam coast, Palk Strait, Southeast coast of India

V. Sri Ramkumar[1*], E. Kannapiran[2] and M. Palanisamy[3]

[1]*School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi, Tamil Nadu, India*
[2]*Department of Zoology and Biotechnology, DDE, Alagappa University, Karaikudi, Tamil Nadu, India*
[3]*Department of Zoology, H.H.Raja's College (Autonomous), Pudukkottai, Tamil Nadu, India*

ABSTRACT

Samples of water and sediment were collected from July to December 2009 in Kottaipattinam coast (Palk Strait, Southeast coast of India). Kottaipattinam coast is one of the major fish landing centre in Palk Strait and receives a considerable amount of sewage and man made waste. Qualitative and quantitative analysis of the composition of the microbial flora were conducted on samples from three stations. The highest bacterial densities, in water and in sediment samples, were found in December and the lowest, in August. Among Gram-negative bacteria, the predominant genus was Pseudomonas; Aeromonas, Vibrio and Flavobacterium were also recorded. Gram-positive bacilli were abundant at all sampling points. Along with physico-chemical parameters were also monitored at every month of sampling.

Key words: Physico-chemical parameters, Total heterotrophic bacteria, Kottaipattinam coast, *Pseudomonas, Aeromonas, Bacillus, Vibrio* and *Flavobacterium.*

INTRODUCTION

Several studies have already been carried out to characterize heterotrophic bacteria in ocean sites and in different coastal areas of temperate, tropical and polar zones [1,2,3,4,5]. Data have been published describing the distribution of bacterial densities which depend on changes in water temperature, salinity, the abundance of organic nutrients, and on other physico-chemical parameters [6,7,8]. However, it has been recognized that bacterial populations may be considerably modified by interactions with biotic factors [9]. In an over-simplified way, the density of bacterial populations in sea water usually ranges from 10^3 to 10^6 ml^{-1}, with counts up

to 10^9 g^{-1} recorded for sediments. In the water column, the presence of micro-organisms usually decreases with increasing depth. Bacterial abundance is also related to the organic matter concentration and to hydrological phenomena such as the interface of water masses with different densities [10,11,12]. Bacteria serve a role analogous to phytoplankton in marine food webs, i.e. they are producers and they are eaten by other organisms. This concept differs substantially from the traditional view that bacteria are the principal agents of mineralization in the sea, acting only slowly on masses of particulate detritus. In fact, the free bacteria sustained on dissolved organic matter assist in rapid mineralization only by serving as prey at the base of a microbial food chain, analogous to the conventional diatomcopepod-fish chain [13].

Horizontal and vertical distribution of bacterial populations in sediments is influenced by various factors, such as the physico-chemical nature of sediments and the presence of high organic matter concentrations. Generally, microbial populations are more abundant in muddy sediments than in sandy ones depending on the granulometry of particles [14]. In aquatic ecosystems, the flux of organic matter to the bottom sediments depends on primary productivity at the ocean surface and on water depth. The number of bacterial cells is usually high where waters are not deep and where there is a large number of organisms; under these conditions, in fact, leaves and other plant and animal residues decay and settle on the bottom before metabolization. This represents a good nutritional substrate for heterotrophic bacteria and favours bacterial growth. In the deep sea characterized by the absence of light, temperatures between 2 and 3°C and a high hydrostatic pressure, the concentration of organic energy is low, which is mirrored by the scantiness of organic residues in sediments. This exerts a negative selective pressure on the microbial flora and consequently, the bacterial concentration in oceans comprises 10^4–10^5 g^{-1}. In sediments, bacteria are present as ‘free-living bacteria’ and associated with organic or mineral particles.

However, heterotrophic bacterial action promotes organic degradation, decomposition and mineralization processes in sediments and in the overlying water, and releases dissolved organic and inorganic substances [15]. The mineralization of organic matter, which is derived from primary producers, results in its being recycled, so that these substances are again available for primary producers. Heterotrophic microorganisms are the major agents shaping the organic composition of the ocean. These heterotrophic bacteria comprise the bulk of microbial populations inhabiting the water column of oceans and are responsible for much of the biological transformation of organic matter and production of carbon dioxide [16]. Distribution of bacteria depends on changes in water temperature, salinity and other physicochemical parameters [17]. Bacteria also serve as an important source of food for a variety of marine organisms. Thus, bacteria not only maintain the pristine nature of the environment, but also serve as biological mediators through their involvement in the biogeochemical processes. In aquatic habitats, the most common bacteria are Gram-negative rods. The majority of the isolates belong to the genera *Pseudomonas*, *Vibrio* and *Flavobacterium*. A higher percentage of Gram-positive bacteria is found in sediments. The present study was carried out to determine the total heterotrophic bacterial community of Kottaipattinam coast (Palk Strait, Southeast coast of India). All the bacteria growing on a Zobell marine agar medium were isolated and identified by several morphological, biochemical and enzymatic tests to the genus level.

MATERIALS AND METHODS

Collection of Samples

Investigations on the Kottaipattinam coast (10°14'16"N and 79°15'59"E), Palk Strait, Southeast coast of India was carried out from July - December 2006. The sampling stations were Kottaipattinam intertidal zone (station 1), Kottaipatinam open sea I (station 2) and Kottaipattinam open sea II (station 3). Seawater and sediment samples were collected respectively from the surface layer of sea water and sediment samples with sediment sampler. All the samples were carried to laboratory as soon as possible at low temperatures. Seawater samples were collected separately for analysis of abiotic factors. Then, 10-fold serial dilutions of the seawater and sediment samples were prepared, using filtered and sterilized 50% seawater.

Bacteriological methods

Serial dilutions of each sample were placed on Zobell marine agar and the viable heterotrophic bacteria were then counted according to the colony-forming unit (CFU) method. The plates were incubated at 28±1°C for 72 hours, and then the colonies were counted. All colonies were isolated, sub-culltured and identified by several morphological, biochemical and cultural methods [18].

RESULTS AND DISCUSSION

Physico-chemical parameters

The basic physical and chemical parameters of the seawater in all the stations were shown in Table 1. From this table, it can be observed that the temperature varied between 24.5 and 32 °C; salinity ranged between 27.5 and 36 ‰; the pH between 7.6 and 8.5 and DO between 3.9 and 8.5. The pH and salinity were positively correlated with temperature and the temperature, pH and salinity were negatively correlated with DO.

Total heterotrophic bacterial population density

The results of bacterial numbers of seawater and sediment samples were shown in table 2. The heterotrophic bacteria occurred at the level of 10^2-10^5 Cfulml^{-1} of surface water and 10^2-10^7 CFU g^{-1} of sediment in all the stations during July – December 2006. The average number of bacteria during the study period was in the order of 10^4 CFU ml^{-1} and 10^6 CFU g^{-1} at all the stations in the Kottaipattinam coast, Palk Strait, although slightly higher values were recorded at station 2 followed by 3 and 1, but in sediment isolation station 3 had higher bacterial population compare to staion 2 and 1. The population density of bacteria in the study area ranged from 3.61to 18.65 x10^4 CFU ml^{-1} in surface waters of all the stations and from 3.5 to14.0x10^6 CFU g^{-1} in the sediments. It is well known that the bacterial concentration in the water column decreases with depth and increases at the sea bottom [19]. According to this rule, the present result envisages that the sediment samples have higher bacterial density than waters. Furthermore, there were some differences between the station 2 and 3 with regard to bacterial concentration. The bacterial load in water and sediment were regatively correlated with abiotic paramenters except DO was positively correlated.

Heterotrophic bacterial flora

A total of 71, 88 and 92 strains of heterotophic bacteria were isolated from the surface water and sediment samples at all the stations respectively. The bacterial floras in all the tested samples were predominated by gram-negative bacteria. In the samples collected from station 1 (Kottaipattinam intertidal zone) 63.5% were gram-negative, station 2 (Kottaipattinam open sea I) 64.73% were Gram-negative and station 3 (Kottaipattinam open Sea II) 65% were gram-negative, remaining 36.5, 35.27 and 35% were gram-positive respectively. From all the isolates were selected to be identified to the genus level. All the gram-negative isolates mainly belonged to 7 genera repeatedly viz. *Pseudemonas, Vibrio, Aeromonas, Flavobacterium, Enterobacter, Cytophaga* and *Alcaligenes*, and the gram-positive isolates belonged to 4 genera viz, *Bacillus, Micrococcus, Arthrobacter* and *Corynebacterium* (Table 3). In aquatic habitats, the most common bacteria are gram-negative rods. The majority of the isolates belong to the genera *Pseudomonas, Vibrio* and *Flavobacterium*. A higher percentage of gram-positive bacteria are found in sediments. *Pseudomonas, Bacillus, Vibrio* and *Aeromonas* were predominant genera from all the 3 stations. Qualitative analysis demonstrated that *Pseudomonas* and *Bacillus* dominates in this environment both in water and sediment, when compare to *Aeromonas*, Aeromonas coming under third dominant genera, Aeromonads are inhabitants of aquatic environments and also belong to the flora of fish, amphibian and other marine organism [20,21,22,23]. *Aeromonas* is predominant in waters with high levels of faecal pollution, and it has therefore been claimed that the presence of Aeromonads can assist in assessments and predictions of aquatic system deterioration or recovery [24,25]. The majority of the isolates in all the station belonged to the genera *Pseudomonas. Pseudomonas* is common in the marine environment and represents a fraction of the total microbial flora characterized by high metabolic versatility, and it is known for its capacity to degrade a considerable amount of synthetic compounds [26]. *Vibrio* is more common in the aquatic habitats and predominant in organic matter in solution and temperature in the mesophilic range. *Flavobacterium* has been isolated from marine water and sediment; it tolerates low, but prefers higher temperatures and can grow in alkaline environments [27]. This genus has been reported to be involved in the degradation of pesticides and chitin. In the marine environments, *Bacillus* is an important gram-positive bacteria and ubiquitous in nature and found waters and sediment samples from polar to tropics [27]. Further more, the high concentration of organic matter could account for the presence of those bacterial genera which characterize a polluted environment.

Table-1 Variations (Range and Annual mean*) of different physico-chemical parameters monitored during July-December, 2007

Station	Temperatute (°C)	pH	Salinity (‰)	DO (ml L^{-1})
Station 1	24.5 – 32 (28.25)	7.6 – 8.2 (7.8)	27.5 – 34.5 (30.75)	3.9 – 5.2 (4.65)
Station 2	25–31.5 (28.5)	7.6 – 8.4 (8.0)	28.5 – 35.7 (32.03)	4.5 – 8.2 (6.25)
Station 3	25.5 – 31.5 (28.67)	7.8 – 8.5 (8.1)	29 – 36 (32.5)	4.72 – 8.5 (6.72)

**Figures in parenthesis indicates mean value*

In conclusion, in Kottaipattinam coast, which is a highly eutrophic coastal ecosystem, most of the autochthonous or allochthonous organic matter could be mireralized in the water column, and at the sediment surface, where different bacterial groups integrate to accomplish complete degradation. The heterotrophic micro-organisms are responsible for the utilization of the extensive pool of dissolved organic carbon (DOC), thus making it available for the different food

webs. In this framework, further studies will be carried out to evaluate the role of each isolated genus in recycling of bioelements, and to further characterize Kottaipattinam coast of Palk Strait, Southeast coast of India.

Table-2 Variations (Range and Annual mean*) of totla heterotrophic bacterial populations during July-December, 2007

Station	Water	Sediment
Station 1	3.61 – 16.5 (10.45)	3.5 – 8.13 (5.93)
Station 2	4.26 – 18.65 (11.65)	7.1 – 12.5 (9.79)
Station 3	4.4 – 18.2 (12.35)	7.94 – 14.0 (10.8)

*Water = No.x10^4 cells ml$^{-1;}$ Sediment = No.x10^6 cells g$^{-1;}$ *Figures in parenthesis indicates mean value*

Table-3 Percentage of bacterial strains present in water and sediment of different stations

Station	Total isolates W/S	(%)		Pse.	Vib.	Aer.	Fla.	Ent.	Cyt.	Alc.	Bac.	Mic.	Arth.	Cory.	Oth.
		G-	G+												
Station 1	32/39	63.5	36.5	17.8	22.3	11.1	6.5	1.6	-	-	14.1	7.7	6.5	3.8	8.6
Station 2	41/47	64.7	35.3	21.2	10.3	8.6	7.5	1.8	2.0	2.7	19.7	8.3	7.1	3.6	7.2
Station 3	43/49	65	35	19.7	4.9	13.2	7.7	3.1	2.2	4.2	15.8	7.9	7.7	3.8	9.8

W/S=water/sediment, G- =Gram negative, G+ =Gram positive, Pse.=Pseudomonas, Vib.=Vibrio, Aer.=Aeromonas, Fla.=Flavobacterium, Ent.=Enterobacter, Cyt.=Cytophaga, Alc.=Alcaligenes, Bac.=Bacillus, Mic.=Micrococcus, Arth.=Arthrobacter, Cory.=Corynebacterium and Oth.=Others.

Acknowledgement

The authors are thankful to the authorities of Alagappa University, Karaikudi and the faculties of the Department of Oceanography and Coastal Area Studies, Thondi Campus for providing necessary facilities to carry out this study.

REFERENCES

[1]. G. Billen, C. Jouris, L.A. Meyer-Reil and H. Lindebloom, *Journal of Sea Research*, **1990**, 26 (2–4), 265–293.
[2]. B.Velimorov and M.Walente-Simon, *Marine Ecology Progress Series*, **1992**, 80, 237–248.
[3]. H.W.Ducklow, D.L. Kirchman, H.L. Quinby, C.A. Carlson and H.G. Dam, **1993**, *Deep Sea Research* 40, 245–263.
[4]. W.J.Wiebe, W.M. Sheldon and L.R. Pomeroy, *Microbial Ecology*, **1993**, 25, 151–159.
[5]. C.S.Hopkinson, B.F. Sherr and W.J. Wiebe, *Marine Ecology Progress Series*, **1998**, 51, 155–166.
[6]. B.Jorgensen and D. Des Marais, *Limnology and Oceanography*, **1988**, 33, 99–113.
[7]. E.Henneke and G.J. de Lange, *Mar Chemistry*, **1990**, 31, 113–122.
[8]. P.Fong, J.Zedler and R.Donohoe Limnology *and Oceanography*, **1993**, 38, 906–923.
[9]. Y.P. Martin and A.Bianchi, *Microbial Ecology*, **1980**, 5, 265–279.
[10]. F.Azam, T.Fenchel, J.G.Field, J.S.Gray, L.A. Meyer-Reil and F.Thingstad, *Marine Ecology Progress Series*, **1983**, 10, 257–263.
[11]. R.La Ferla, and E.Crisafi, *Marine Ecology Progress Series*, **1991**, 75, 309–311.
[12]. A.Bianchi and J.Garcin, *Deep Sea Research*, **1993**, 40, 1703–1710.

[13]. H.W. Ducklow, *BioScience,* **1983**, 33, 494–501.
[14]. P.Lakshmanaperumalsamy, D. Chandramohan and R.Natarajan, In *Abstracts Gerbam 2e`me Colloque International de Bacte´riologie Marine,* CNRS, Brest, **1986**, Vol 3.
[15]. A.Purushothaman, In Proceedings of the Technical Workshop on Biodiversity of Gulf of Mannar Marine Biosphere Reserve, M. S. Swaminathan Research Foundation, Chennai, **1998**, pp. 86–91.
[16]. E. B. Sherr and B. F.Sherr, *Aquat. Microb. Ecol*., **1996**, 1, 91–100.
[17]. S. V.Alavandi, *Indian J. Mar. Sci*., **1990**, 30, 89–92.
[18]. J.G. Holt, N.R. Krieg, D.H.A. Sheath, J.T. Stanley and S.T. Williams, *Bergey's manual of determinative bacteriology,* 9th Edn., **1994**, 787 pp.
[19]. A. Bianchi, In *Aquatic Microbial Ecology* ed. R.R. Colwell and J.A. Foster, **1980**, pp. 372–376. University of Maryland, College Park: Maryland Sea Grant Publication.
[20]. E.B.Shotts, J.L.Gaines, C.Martin and A.K. Prestwood, *Journal of the American Veterinary Medicine Association,* **1972**, 161, 603–707.
[21]. J.B. Kaper, H.Lockman and R.R. Colwell, *Journal of Applied Bacteriology,* **1981**, 50, 359–377.
[22]. C.S. Kueh and K.Y. Chan, *Journal of Applied Bacteriology,* **1985**, 59, 41–47.
[23]. R.A. Cavallo, C. Rizzi, T. Vozza and L. Stabili, Journal of Applied Microbiology, **1999**, 86, 906–916.
[24]. K.Venkateswaran and R.Natarajan, *Indian Journal of Marine Science,* **1987**, 16, 51–53.
[25]. R.M.Araujo, R.M. Arribas and R.Pares, *Journal of Applied Bacteriology,* **1991**, 71, 182–186.
[26]. S.Nair and U. Simidu, *Applied and Environmental Microbiology*, **1987**, 53, 2957–2962.
[27]. H.Stolp, *Microbial Ecology. Organisms, Habitats, Activities.* Cambridge: Cambridge University Press, **1988**.