

Short Communication

Density of heterotrophic bacteria in Meghadri mangrove ecosystem, Visakhapatnam, east coast of India

*G. Raghavendrudu and B. Kondalarao

Department of Marine Living Resources, Andhra University, Visakhapatnam- 530003, Andhra Pradesh, India.*E-mail: raghuvenkat@sify.com

Abstract

The density of heterotrophic bacteria was investigated from the sediments of Meghadri mangrove ecosystem of Visakhapatnam from April 2006 to March 2007. The sediments harboured the bacterial genera *Bacillus, Pseudomonas, Brevibacterium, Staphylococcus* and *Micrococcus*. The mean density of total heterotrophic bacteria was 7.93 x 10³cfu.g⁻¹. Among the proteolytic, glycolytic and lipolytic bacterial densities, the lipolytic bacteria (5.31 x 10³ cfu.g⁻¹) were found higher than the other bacteria. The mean values of temperature, salinity, pH, dissolved oxygen and sedimentary organic matter during the study period were 29.0°C, 33.1 ppt, 7.5, 2.27 mg/ml and 2.13% respectively. The density of bacteria in the mangrove sediments is discussed in relation to the physico-chemical parameters.

Mangrove ecosystems support a wide variety of organisms, of which, microbial populations specifically bacteria and fungi play an important role in remineralisation and production of biologically active substances like antibiotics, vitamins, enzymes, amino acids, alcohols etc. Harold and Millwood (1974), Droop and Jannasch (1977) and Chandra Mohan (1997) investigated the heterotrophic bacteria from the estuarine and coastal habitats. The Meghadri River in Visakhapatnam (east coast of India) harbours small patches of mangrove vegetation (17° 42' N lat; 83° 16'E long.) principally composed of Avicennia marina and Excoecaria agallocha. There are several studies on the distribution and density of bacteria in the Indian mangrove habitats (Choudhary, 1987; Ramamurthy et al., 1990; Rajeswari et al., 1995; Ratnakala and Chandrika, 1997; Untawale and Wafar, 1997; Priya et al., 2004; Surajitdas et al., 2007). However, information on the density of heterotrophic bacteria from Andhra Pradesh mangrove habitats is limited. We made an attempt to study the distribution of heterotrophic bacteria with emphasis on proteolytic, glycolytic and lipolytic bacterial populations from Meghadri mangroves of Visakhapatnam port.

Materials and methods

Sediment samples were collected at fortnightly intervals during April 2006 - March 2007 from six selected sites of Meghadri mangroves. Sediment core samples from the study area were aseptically collected in sterile polythene bags. Each sediment sample (10g) was diluted with 100 ml of aged sterile seawater (25% distilled water and 75% seawater). The inoculations were prepared aseptically using ten fold dilutions. The total heterotrophic bacteria, glycolytic bacteria, proteolytic bacteria and lipolytic bacteria were cultured on Zobell marine agar (Zobell, 1946), carbohydrate medium (Rhodes medium; Aaronson, 1970), Frazier gelatin agar (Weyland et al., 1970) and lipolytic medium (Sierra, 1957) respectively, at 33°C for 24 to 36 hours under sterile conditions. The glycolytic, proteolytic, and lipolytic bacterial populations were identified by processing the cultures with gram iodine, saturated ammonium sulphate and saturated copper sulphate solutions respectively. The colonies were counted using a bacteriological colony counter and the densities were expressed as 103 cfu.g-1. Bacteria grown on the cultures were identified up to genus level using the culture, morphological and biochemical

Table 1. Physico-chemical parameters and density of bacterial populations in Meghadri mangrove sediments during April 2006 - March 2007

Months	T(°C)	S(ppt)	D.O (mg/ml)	pН	S.O.M (%)	T.H.B	G.B	P.B	L.B
Apr I	32.0	34.2	2.24	7.4	1.80	10.0	3.4	3.2	6.6
Apr II	29.5	35.2	2.48	7.8	1.94	7.8	3.5	6.0	7.5
May I	26.4	34.0	2.80	7.5	2.10	13.3	6.5	8.3	7.0
May II	28.5	35.0	2.50	7.5	2.25	8.2	6.6	6.4	6.9
Jun I	30.4	35.4	1.89	7.5	1.69	7.8	4.8	5.0	6.0
Jun II	29.1	35.0	2.80	7.5	1.94	9.2	4.8	4.7	5.3
Jul I	26.5	32.6	1.80	7.5	2.26	8.8	4.2	4.5	5.2
Jul II	28.8	35.4	2.50	7.1	2.05	7.4	5.2	5.0	6.6
Aug I	30.2	36.3	1.87	7.8	2.00	8.2	5.2	6.3	5.2
Aug II	27.5	35.6	1.38	7.5	1.95	5.8	2.1	3.2	3.7
Sep I	29.5	34.5	1.68	7.5	1.99	8.2	3.2	4.1	5.3
Sep II	28.7	27.5	1.49	7.7	1.79	7.6	4.8	5.2	5.0
Oct I	28.4	34.5	2.80	7.5	2.50	7.8	3.2	3.5	4.5
Oct II	-	-	-	-	-	-	-	-	-
Nov I	24.3	35.6	1.81	7.4	1.80	8.0	4.8	5.4	6.0
Nov II	29.9	33.2	2.80	7.5	2.50	9.0	5.7	6.9	6.6
Dec I	28.5	34.5	3.00	7.6	2.40	7.8	4.7	4.1	5.5
Dec II	29.5	29.5	2.10	7.5	1.80	4.2	3.2	3.1	3.9
Jan I	30.6	28.4	2.50	7.5	2.40	6.4	5.0	4.2	4.6
Jan II	30.0	30.0	2.20	7.8	2.20	5.4	3.4	3.6	4.0
Feb I	30.4	32.0	2.60	7.5	2.60	10.4	5.4	4.4	4.8
Feb II	30.6	30.2	2.40	8.0	2.30	6.0	5.0	4.8	4.2
Mar I	30.0	30.0	2.00	7.8	2.20	6.4	4.0	3.8	3.4
Mar II	28.8	32.2	2.60	8.0	2.60	8.8	3.6	2.8	4.4
Mean	29.0	33.0	2.27	7.5	2.13	7.93	4.4	4.71	5.31

(T = Temperature, S = salinity, D.O = dissolved oxygen, S.O.M = sedimentary organic matter, T.H.B = total heterotrophic bacteria, G.B = glycolytic bacteria, P .B = proteolytic bacteria, L.B = lipolytic bacteria; bacterial counts are 10^3 cfu.g $^{-1}$; - denotes no sample)

Table 2. Pearson correlations between physicochemical parameters and bacterial densities (n= 23) in Meghadri mangrove sediments

Bacteria	Temperature	Salinity	Dissolved oxygen	рН	Soil organic matter	
T.H.B	-0.19914*	0.379618*	0.366966*	-0.21131*	0.145035*	
G.B	-0.09894	0.049686	0.408683	-0.15507*	0.193876*	
P.B	-0.26421*	0.284564*	0.205968*	-0.10708	-0.07744*	
L.B	-0.13898*	0.547981*	0.308024*	-0.40169*	-0.2149*z	

(* indicates significance at p < 0.05). (T.H.B = total heterotrophic bacteria, G.B = glycolytic bacteria, P.B = proteolytic bacteria, L.B = lipolytic bacteria)

Journal of the Marine Biological Association of India (2008)

characters (Skinner, 1975). Sediment temperature was recorded using a 0.1°C sensitivity thermometer. Salinity and dissolved oxygen of sediment water samples were measured by Knudsen method and Winkler's method (Strickland and Parsons, 1965). The sediment organic matter was determined by chromic acid digestion method (Jackson, 1967) and sediment pH by a digital pH meter (Elico). The density data were processed using SPSS software Version 10.

Results and Discussion

During the present study, the mangrove sediments harboured five genera of bacteria namely, Bacillus, Brevibacterium, Staphylococcus, Pseudomonas and Micrococcus. The first four genera were regularly recorded in the sediments. Micrococcus was recorded only in April I, September II and February II samples. Choudhary (1987) reported eight genera of bacteria from Sunderban mangrove sediments. Four genera, i.e., Bacillus, Brevibacterium, Pseudomonas and Micrococcus are present in both the Sunderbans Meghadri mangroves. The Brevibacterium is reported from the east coast mangroves only namely, Sunderbans and Meghadri mangroves. Rajeswari et al. (1995) reported twelve genera of bacteria from Andaman mangrove sediments. Priya et al. (2004) recorded twelve genera of bacteria from Zuari mangrove sediments. The qualitative data indicate that the genera Bacillus, Pseudomonas, Staphylococcus and Micrococcus are the common heterotrophic bacteria in the mangrove habitats. The mean values of temperature, salinity, pH, dissolved oxygen and sediment organic matter of the Meghadri mangroves were 29.0° C, 33.1 ppt, 7.5, 2.27 mg/l and 2.13% respectively. The mean densities of glycolytic, proteolytic, lipolytic and total heterotrophic bacteria recorded were 4.40, 4.71, 5.31 and 7.93 x 10^3 cfu.g⁻¹ respectively (Table 1). The density of lipolytic bacteria was relatively higher in the sediments which may be attributed to the availability of lipid sources in the ecosystem due to the hydrocarbons released from the adjacently located HPCL oil refinery. The seasonal density of bacteria exhibited almost similar trend

with peak abundance during May (summer) and late November (winter). The seasonal density fluctuations were more prominent in the total heterotrophic bacteria, glycolytic bacteria and proteolytic bacteria, but negligible in lipolytic bacteria. Surajitdas et al. (2007) reported 1.01 x 103 to 37.98 x 103 cfu.g-1 heterotrophic bacteria in 10³ the slope sediments of western Bay of Bengal. The density of mangrove sediment bacteria of Mandovi estuary ranged from 2.65x10³ cfu.g⁻¹ in premonsoon to 3.56 x 10³ cfu.g⁻¹ in monsoon (Priya et al., 2004). Table 2 provides Pearson correlations between physico-chemical parameters and bacterial densities in the Meghadri mangrove sediments. The positive correlations of salinity and dissolved oxygen with total heterotrophic bacteria, proteolytic bacteria and lipolytic bacteria were significant (p < 0.05). The negative correlations of temperature with total heterotrophic bacteria, proteolytic bacteria and lipolytic bacteria and of pH with total heterotrophic bacteria, glycolytic bacteria and lipolytic bacteria were observed as significant (p < 0.05). The soil organic matter showed positive correlations with total heterotrophic bacteria and glycolytic bacteria but negative correlation with proteolytic bacteria and lipolytic bacteria. The correlation analysis indicates the importance of soil organic matter in the density of the four groups of bacteria. In conclusion, it may be stated that the heterotrophic bacteria of Meghadri mangrove sediments registered seasonal differences, and the community consisted of relatively higher density of lipolytic bacteria when compared to the other components.

Acknowledgements

The authors are thankful to Andhra University for providing laboratory facilities.

References

Aaronson, S. 1970. Experimental Microbial Ecology.
Academic press, New York, 231 pp.

Chandra Mohan, D. 1997. Recent advances in marine microbiology: The Indian scenario. J. Mar. Biotechnol., 5: 73-81.

Choudhary, A. 1987. *Multidisciplinary research project on mangroves of Sundarbans*. A project report. University of Calcutta, 87 pp.

- Droop, M. R. and H. W. Jannasch. 1977. *Advances in Aquatic Microbiology*. Academic press, New York, 213 pp.
- Harold, S. L. and C. E. Millwood. 1974. Aerobic, heterotrophic bacterial populations in estuarine water and sediments. In: R. R. Colwell and R.Y. Morita (Eds). Effect of the Ocean Environment on Microbiol Activities, University Park Press, Baltimore, U.S.A., p. 268-285.
- Jackson, M. L. 1967. Soil Chemical Analysis. Prentice Hall of India Ltd, New Delhi. 498 pp.
- Priya, M. D., S. Kalekar and S. Bhosle. 2004. Diversity of free-living and adhered bacteria from mangrove swamps. *Indian J. Microbiol.*, 44: 247-250.
- Rajeswari, S., B. R. Shome, A. B. Mandal and A. K. Bandopadhyay. 1995. Bacterial flora in mangroves of Andaman- Part I: Isolation, identification and antibiogram studies. *Indian J. Mar. Sci.*, 24: 97-98.
- Ramamurthy, T. R., Mohanraju and R. Natarajan. 1990. Distribution and ecology of methanogenic bacteria in mangrove sediments of Pitchavaram, east coast of India. *Indian J. Mar. Sci.*, 19: 269-273.
- Ratnakala, R. and V. Chandrika. 1997. Microbial production of antibiotics from mangrove ecosystem. CMFRI Spl. Publ., 61:117-122.
- Sierra, G. 1957. A simple method for the detection of lipolytic activity of microorganisms and some

- observations on the influence of the contact between cells and fatty substances. *Antonne Van Leeuwenhoek*, 23: 15-23.
- Skinner, A. 1975. *Identification Methods for Microbiologists*, Academic press, New York, 230 pp.
- Strickland, J. D. H. and T. R. Parsons. 1965. Practical Manual of Sea Water Analysis. Bull. Fish. Res. Bd. Canada, 167: 251 pp.
- Surajitdas, S., P. S. Lyla and S. Ajmalkhan. 2007. Spatial variation of aerobic cultural heterotrophic bacterial population in sediments of the continental slope of western Bay of Bengal. *Indian J. Mar. Sci.*, 36: 51 58.
- Untawale, A. G. and M. Wafar. 1997. Litter fall energy flux in a mangrove ecosystem, Mandovi-Zuari estuary. Estuar. Coast. Shelf Sci., 44:111-124.
- Weyland, H., H. J. Ruger and H. Schwarz. 1970. Zur isolierung und identifizie rung mariner bakterian. Ein beitrangzur standrdisierung und entwicklung daquater methoden. Veroff. Inst. Meeresforch. 12: 269-296.
- Zobell, C. E. 1946. *Marine Microbiology*, Chronica Botanica Co., Waltham, Massachusetts, U.S.A., 240 pp.

Received: 29 November 2007 Accepted: 9 April 2008