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Biochemical Characterization of Total HeterotrophicBacteria (THB) in Muthukuda Mangroves, Southeast Coast of India

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Abstract: Heterotrophic bacteria and their processes in the mangrove environment in to focus, an understanding on their abundance, distribution, production and, their involvement in nutrient cycling and how they are at the base of microbial food web is essential. The first time Total Heterotrophic Bacteria (THB) was Screened from Muthukuda mangrove sediment. Eight isolate were selected based on the colony morphology and of colonies were identified by phenotypic and biochemical character such as *Bacillus subtilis, Streptococcus sp. Staphylococcus sp. Carnybacterium sp. Photobacterium sp. Enterobacteriaceae sp. Escherichia coli* and *Actinobacteria sp*.

Key words: Mangrove · Biochemical Characterization · Heterotrophic bacteria · Phenotypic character

INTRODUCTION

In this largest marine habitat mangroves are the most productive ecosystem. Mangrove ecosystems are dominant ecosystems along tropical coastlines. In India, the total area covered by mangroves over 4,900km, which accounts 8% of Indian total coastline. In tropical mangroves, bacteria and fungi constitute 91% of the total microbial biomass, whereas algae and protozoa represent only 7% and 2% respectively [1]. The marine environment is characterized by the hostile parameters such as high pressure, salinity, low temperature, absence of light, etc. and marine heterotrophic bacteria have adapted themselves to survive in this environment they require Na^+ for growth because it is essential to maintain the osmotic environment for protection of cellular integrity and also the 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 [2].

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 [3]. Heterotrophic bacteria as an important component in the absorption of light in marine environments [4, 5]. A large number of heterotrophic bacteria can synthesize Carotenoids and Carotenoid-rich species have been isolated from both coastal and oceanic waters in recent years [6]. The very basic functional characteristic of decomposition by heterotrophic bacteria is of considerable ecological and economic importance in pollution clean-up. In the present study focus on the distribution and biochemical characterization of heterotrophic bacteria in Muthukuda mangrove sediment.

MATERIALS AND METHODS

Description of Study Area: Muthukuda is located (Lat. 9°54’ 10.20”N; Long. 79°09’07.13”E) 20 km north of Thondi lies along the south eastcoast of India. Muthukuda mangrove covers an total area of approximately 12ha. In this Mangrove, *Avicennia marina i*s dominant species in this coastal region. Seagrasses *Cymodocea serrulata, Syringodium isoetifolium, Halodule pinifolia* is dominanted by this region. Mangroves sediment are acidic and clay in nature. Sediment becomes loose because of the presence of the decaying organic matter and sediment is black in colour. Fishing activities are high in this coastal region by using mechanical and non mechanical boats regularly.

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Sample Collection: Sediment sample were collected from the Muthukuda Mangroves, Southeast coast of India. The rhizosphere sediment samples were collected in the clean polyethylene bags and transported to the laboratory by keeping them in ice box and processed within 3 hours and microbial analysis were carried within 4 hours.

Screening and Identification of Total Heterotrophic Bacteria (THB): The sediment sample were serially diluted and plated in Zobell 2216 marine agar medium [7] prepared with 50% aged seawater [8]. Triplicate plates from each dilution were incubated at 28ºC. After incubation the colonies were counted by colony forming unit (CFU) and subculture by colony morphology. The different morphological and biochemical characterization [9] of the isolates were investigated according to the Bergey's Manual of determinative bacteriology [10].

RESULTS AND DISCUSSION

Phenotypic Characterization: Bacteria inhabiting mangroves environment are dominant microorganisms, fairly well adapted to the extreme condition of mangrove ecosystem. Recent bacteriological studies of mangrove environment concerned mainly their sanitary, pollution and bacterial number [11]. Eight different isolates were identified and sub cultured in the Marine Agar medium (Table 1). The following isolate 1, 2, 3 and 8 observed as round colonies, isolate 5 is transparent in nature, Rhizoid structure was observed in the isolate 4, isolate 6 was Mucoid and isolate 7 was filamentous in nature. Colony size of the isolates also varied from the range of 0.1- 1.5mm. The colour was also varied as pink, orange, yellow white and pale white.

Biochemical Characterization: There are many methods for identifying bacteria. Traditionally, an observational and biochemical approach has been used. Simply looking at (and even smelling) a bacterial colony growing on an agar plate can give an experienced researcher clues to a bacterium's identity. Bacteria are categorized as "Gram Positive" or "Gram Negative" according to whether or not they are stained by a chemical dye, a common biochemical technique [9]. The screened isolates were subjected in to the biochemical characterization, test results of Indole, Methyl red, Voges proskaur, citrate utilization, carbohydrate fermentation (Glucose, Sucrose and Lactose) and Catalase, oxidase (Table-2).

The results obtained in this study portray the bacterial community associated to the mangrove rhizosphere as a dynamic one, experiencing important changes in abundance of both total and active bacteria. In contrast to the vertical exponential decline in the bacterial abundance often observed in muddy terrigenous sediments [12-14] the community analyzed here was, in terms of abundance, rather homogeneous through the depth. The results from our study indicate a higher proportion of gram-negative bacteria than gram-positive bacteria among the species of heterotrophic bacteria. The results were in agreement with the general rules that the proportion of gram negative bacteria is much higher than the proportion of gram-positive bacteria in the ocean [15-17].

In our study it indicated that *Actinobacteria sp. Bacillus subtilis, Carnybacterium sp. Enterobacteriaceae sp. Escherichia coli Streptococcus sp. Staphylococcus sp.* and *Photobacterium sp.* were abundant in the mangrove rhizosphere sediment samples. The result of *Streptococcus sp.* and *Escherichia coli* were comparable to the earlier reports of Thompson [18] recorded 9 genera and Paramasivam [19] recorded 10 genera of THB from Pitchavaram and Muthupettai mangrove environment, respectively. Bacteria belonging to the genus of *Staphylococ*cus *sp*. have been found attached to leaves, rhizomes and roots [20]. The same bacterial genera have been isolated from marine and estuarine surface water samples Gunn and Colwell [21]. According to Kannan and Vasantha [22] the presence of *Acintetobacter sp.* the combined effect of low salinity and light extinction at the fresh water zone promtes the occurrence of gram positive bacteria when compare to marine zone. The *Actinobacteria* bacteria are primarily saprophytic and are best known from soils, where they contribute significantly to the turnover of complex biopolymers such as lignocellulose, hemicellulose, pectin, keratin and chitin [23, 24]. Several groups of *Actinobacteria*, including species from the genera *Micrococcus* and *Corynebacterium*, can synthesize cyclic and acyclic C45 and C50 carotenoids [25, 26]. *Actinobacteria* bacteria are more competitive in degrading organic matter or in growing on agar plate than *Bacteroidetes* bacteria, which was revealed by a previous study by Sekiguchi [27].

Table 1: Showing the Morphological Characters of different bacterial isolates

Isolates	Colony Colour	Colony Size (mm)	Morphology and Nature of the Colony
1	Pink	0.1- 0.3	Round
2	Orange	0.1-0.5	Round
3	Yellow	0.5- 1.5	Round, convex
4	White	0.5- 1.0	Rhizoid, sticky
5	White	0.5- 1.2	Transparent
6	Pale white	0.3- 0.7	Mucoid, circular
7	White	0.7- 1.5	Filamentous, irregular
8	white	0.1- 0.6	Round, convex

Table 2: Phenotypic and Biochemical Characteristics of Isolates

	Isolates							
Tests	1	2	3	4	5	6	7	8
Gram Staining	+ Ve Rod	-Ve Cocci	+ Ve Rod	-Ve Rod	-Ve Rod	-Ve Cocci	+Ve Cocci	-Ve Rod
Indole	+	+	+	+	+	+	+	+
Methyl red	+	+	+	+	+	+	+	+
Voges proskaur	-	-	-	+	-	-	-	+
Citrate utilization	-	-	-	-	+	-	-	-
Glucose	+	+	+	+	+	+	+	-
Sucrose	-	-	-	+	-	+	-	+
Lactose	-	-	-	+	-	-	-	-
Catalyse	+	+	+	+	+	+	+	+
Oxidase	-	-	-	+	-	-	-	-
Starch hydrolysis	+	-	-	+	+	-	-	-
Nitrate reduction	+	+	+	+	+	+	+	+

+ve= Positive; -ve= Negative

The genus *Bacillus* comprises a phylogenetically and phenotypically heterogeneous group of species. Due to their ubiquity and capability to survive under adverse conditions, heterotrophic *Bacillus* strains are hardly considered to be species of certain habitats [28]. However, it is generally accepted that the primary habitat of the aerobic endospore-forming Bacilli is the soil. Since most *Bacillus* species can effectively degrade a series of biopolymers (proteins, starch, pectin, etc.) they are assumed to play a significant role in the biological cycles of carbon and nitrogen. Several *Bacillus* strains from soils and mangrove sediments have already been reported as hydrocarbon degraders and emulsifier producers Holguin [29]. Macrae [30] found bacilli as dominant rhizosphere organisms in mangroves and suggested that they should be targeted to provide microbial solutions which ameliorate polluted environments.

CONCLUSION

The present study concluded that the phenotypic and biochemical analysis is a suitable tool for characterize the Total Heterotrophic Bacterial (THB) community in Muthukuda mangrove sediment and better understand the functioning of their related ecosystems. In addition to their distribution pattern and involved in nutrient cycling how they are act as a tool for biodegrading th nutrients in food web.

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REFERENCES

1. Alongi, D.M., 1988. Bacterial productivity and microbial biomass in tropical mangrove sediments. Microb Ecol., 15: 59-79.
2. Sherr, E.B. and B.F. Sherr, 1996. Temporal offset in oceanic production and respiration process implied by seasonal changes in atmospheric oxygen: The role of heterotrophic microbes: Aqua Microbiol Ecol., 1: 91-100.
3. Purushothaman, A., 1998. Microbial diversity. In Proceedings of the Technical Workshop on Biodiversity of Gulf of Mannar Marine Biosphere Reserve, M. S. Swaminathan Research Foundation, Chennai., pp: 86-91.

4. Morel, A. and Y.H. Ahn, 1990. Optical efficiency factors of free-living marine bacteria: influence of bacterioplankton upon the optical properties and particulate organic carbon in oceanic waters: J. Mar. Res., 48: 145-175.
5. Stramski, D. and D.A. Kiefer, 1991. Light scattering by microorganisms in the open ocean: Prog Oceanog., 28: 343-383.
6. Yurkov, V.V. and J.T. Beatty, 1998. Aerobic an oxygenic phototrophic bacteria: Microbiol. Mol. Biol. Rev., 62: 695-724.
7. Rheinheimer, G., 1997. Microbial ecology of brackish water environment: Ecol. Stud., 25: 39-60.
8. Pugazhven, S.R., S. Kumaran, K. M. Alagappan and S. Guruprasad, 2010. Inhibition of fish Bacterial pathogens by antagonistic marine actinomycetes: European J. Appl. Scie., 2(2): 41-43.
9. Mahalakshmi, M., M. Srinivasan, M. Murugan, S. Balakrishnan and K. Devanathan, 2011. Isolation and identification of total heterotrophic bacteria and human pathogens in water and sediment from Cuddalore fishing harbour after the tsunami: Asian J. Biolol. Scie., 4: 148-156.
10. Holt, G.H., N.R. Kreig, P.H.A. Sneath, J.T. Staley and Willams, 1997. Bergey's Manual of Determinative Bacteriology. 9 Th Ed. Willams and Wilkins, New York.
11. Papadakis, J.A., A. Mavridou, S.C Richardson and M. Lampiri, U. Marcelou, 1997. Bacther-related microbial and yeast populations in sand and seawater: Water Res., 31: 799-804.
12. Llobet-Brossa, E., R. Rosselló-Mora, R.I. Amann, 1998. Microbial community composition of Wadden Sea sediment as revealed by fluorescence in situ hybridization. Appl. and Environ Microbiol., 64: 2691-2696.
13. Rosselló-Mora, R., B. Thandrup, H. Shcäfer, R. Weller and R.I. Amann, 1999. The response of the microbial communities of marine sediments to organic carbon input under anaerobic conditions. Systematic and Appl. Microbiol., 22: 237-248.
14. Morel. A. and Y.H. Ahn, 1990. Optical efficiency factors of free-living marine bacteria: influence of bacterioplankton upon the optical properties and particulate organic carbon in oceanic waters. J. Mar. Res., 48: 145-175.
15. Haglund, A.L., P. Lantz, E. Tornblom and L. Tranvik, 2003. Depth distribution of active bacteria and bacterial activity in lake sediment. FEMS Microbiol. Ecol., 46: 31-38.
16. Austin, B., 1988. Marine Microbiol., Cambridge: Cambridge University Press.
17. Oliver, J.D., 1982. Taxonomic scheme for the identification of marine bacteria. Deep Sea Reser., 29: 795-798.
18. Jensen, P.R. and W. Fenical, 1995. The relative abundance and seawater requirements of gram-positive bacteria in near-shore tropical marine samples. Microb. Ecol., 29: 249-257.
19. Thompson, F., L.T. lida and J. Swings, 2004. Biodiversity of vibrios. Microbiol. Mol. Biol. Rev., 68: 403-431.
20. Paramasivam, S., 2002. Studies on histamine producing bacteria of mangrove environment of Muthupettai (Southeast coast of India). Ph.D Thesis, Annamali University, India. pp: 93.
21. Wahbeh, M.L. and A.M. Mahasneh, 1984. Heterotrophic bacteria attached to leaves, rhizomes and roots of three seagrass species from Aqaba (Jordan). Aquat Bot., 20: 87-96.
22. Gunn, B.A. and R.R. Colwell, 1983. Numerical taxonomy of *Staphylococci* isolated from the marine environment. Int. J. Syst Bacteriol., 33: 751-759.
23. Kannan, L. and K. Vasantha, 1986. Distribution of heterotrophic bacteria in velar estuary, east cost of India. Indian J. Mar. Sci., 15: 267-268.
24. Williams, S.T., S. Lanning and E.M.H. Wellington, 1984. Ecology of Actinomycetes. The Biology of the Actinomycetes, (Goodfellow M, Mordarski M and Williams ST, ed.). 481-528. Academic Press, London, UK.
25. Stackebrandt, E., F.A. Rainey and N.L. Ward-Rainey, 1997. Proposal for a new hierarchic classification system, Actinobacteria classis nov. Int. J. Syst. Bacteriol., 47: 479-491.
26. Goodwin, T.W., 1980. The Biochemistry of the Carotenoids. Plants. 2nd edn. Chapman and Hall, New York.
27. Sekiguchi, H., H. Koshikawa, M. Hiroki, S. Murakami, K. Xu, M. Watanabe, T. Nakahara, M. Zhu and H. Uchiyama, 2002. Bacterial distribution and phylogenetic diversity in the Changjiang estuary before the construction of the Three Gorges Dam. Microb. Ecol., 43: 82-91.
28. Claus, D. and R.C.W. Berkeley, 1986. Genus *Bacillus*, Cohn, 1872. P. H. A. Sneath, N. S Mair, M. E. Sharpe, J. G Holt JG (Edn.) Bergey's Manual of Systematic Bacteriology. Vol. 2. Baltimore: The Williams and Wilkins Co., pp: 1105-1139.

29. Holguin, G., P. Vazquez and Y. Bashan, 2001. The role of sediment microorganisms in the productivity, conservation and rehabilitation of mangrove ecosystems: an overview. Biol Fertil Soils., 33: 265-278.

30. Macrae, A., C.M.M. Lucon, D.L. Rimmer and A.G. O'Donnell, 2001. Sampling DNA from the rhizosphere of Brassica napus to investigate rhizobacterial community structure. Plant Soil., 233: 223-230.