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Bacterial Diversity of Mangrove Soil in Karankadu from East Coast of Tamil Nadu, India

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A B S T R A C T

In the present investigation about bacterial diversity from mangrove soil of Tamilnadu in four seasons were analyzed. Bacterial isolation was done by the soil dilution method incubated at 37°C for 24 hours. Totally twenty-seven different bacterial colonies were isolated from Karankadu mangrove soil. The maximum bacterial colonies were presented in summer seasons were compare with pre-monsoon, monsoon and post-monsoon seasons. The predominant bacterial genera namely *Micrococcus spp.*, *Bacillus* spp., *Acetobacter* spp., *Pseudomonas* spp., *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Sulfidobacillus* spp., *Escherichia coli.*, *Aeromonas* spp., *Brevibacterium* spp., *Listeria* spp., *Azotobacter* spp., *Cellulomonas* spp., *Corynebacterium* spp., *Aerococcus* spp., *Klebsiella* spp., *Marinococcus* spp., *Enterobacter* spp., *Thiobacillus* spp., *Planococcus* spp and *Shigella* spp.

Introduction

Mangrove forests are distributed throughout the tropical and subtropical coasts of the world. They are particularly well developed in estuarine areas of the tropics, where they reach their greatest areal extent. The World's mangroves span over 30 countries with a total area of 99,300 sq. km. The largest mangrove area occurs in Indonesia (30%), Brazil (10%), Australia (8%), India and Nigeria (7%, each). World-wide mangroves are disappearing at an alarming rate. In some developing countries about 80% of mangroves were lost in the last three decades. Mangrove environments are unique atmosphere, harboring diverse groups of microorganisms which perform an important role in nutrient cycling and regulate chemical environment of the ecosystem (Alongi *et al.*, 1993; Holguin *et al.*, 1999). The free living bacteria, fungi and yeasts were reported to have significant role in formation of accrual in the mangrove ecosystems (Maria and Sridhar, 2002). The phylogenetic and functional description of microbial diversity in the mangrove ecosystem has not been well addressed to the same extent as that of the other

environments (Zhou *et al*., 2006). The microbial diversity and distribution in a mangrove would improve our understanding of bacterial functionality and their interactions found in that ecosystem (Kathiresan and Selvam, 2006). Hence, in the present study investigated to assess the bacterial diversity from mangrove soil of Tamilnadu in different seasons to understand the structural and functional dynamics in the mangrove ecosystem and to identify some biotechnologically important bacteria for their further application.

Materials and Methods

Sampling Schedule

Soil samples were collected from karankadu mangrove soil in four seasons for a period of 2013-2014**.**

Bacterial Analysis

Soil samples were processed for isolation of bacteria. One gram sample was used for each inoculums. Serially diluted inoculums (10^{-4} to 10^{-6}) were used for inoculums in pore-plating techniques for isolation of bacteria.

Isolation of Pure Culture and Identification

The colonies growing on nutrient agar plates with different morphology were counted separately. The different bacterial colonies from the mother culture were picked up by sterile inoculation loop and aseptically inoculated into the separate nutrient agar plates. These plates were incubated at 37±2°C for 24 hrs and each plate contain single kind of bacteria. The isolated bacteria strains were identified based on their cultural morphological and biochemical characteristics (Cappuccino and Sherman, 1999).

Results and Discussion

The bacterial diversity of mangrove soil in different seasons results were studied (table –1). Totally twenty-seven different bacterial colonies were isolated in various seasons. The maximum bacterial colonies were presented in summer seasons pre-monsoon, monsoon and post-monsoon seasons. The importance of bacterial generated detritus in mangrove areas that acts as the major substrate for bacterial growth in mangrove ecosystems was outlined in a conceptual model by Bano and Nisa (1997).

The morphologically, isolated bacterial colonies were observed round, oval, translucent, irregular and the colour was also indicated as orange, yellow, white, pink (Table-2).

The study of marine bacterial diversity is important in order to understand the community structure and pattern of distribution. Bacteria are generally less than 1–2µm in size except for the largest bacterium *Epulopiscium fishlesoni*, a Gram-positive species 200 – 800µm long that lives in the gut of a Red Sea fish (Fell *et al*., 1984). In the marine environment, 90% of bacteria are Gram-negative with different characteristics (Zobell, 1946) and the Gram-negative cell wall is better adapted for survival in the marine environment but contradictory utmost of bacteria Gram-positive in mangrove soil. Eighteen bacterial flora isolates that metabolize waste drilling fluid were collected from a mangrove swamp in Nigeria (Oliver, 1982).

The bacterial strains are identified an observational and biochemical approach has been used (Table-3). Interestingly, four additional bacterial strains isolated from the same swamp depress growth rates of *Staphylococcus* and *Pseudomonas* species and could, therefore, decrease normal rates

of organic decomposition (Ganesh Babu *et al*., 2004). Similarly same isolates were observed from mangrove soil in different seasons. Certain bacterial strains such as *Pseudomonas mesophilica*, *P. caryophylls* and *Bacillus cereus* exhibit magnetic behavior which may be called magnetobacteria isolated from mangrove sediments of Pichavaram, Southeast India (Bernard *et al*., 2000).

In the present study totally twenty-seven bacterial species were identified such as *Micrococcus* spp., *Bacillus* spp., *Acetobacter* spp., *Pseudomonas* spp., *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Sulfobacillus* spp., *Escherichia coli*., *Aeromonas* spp., *Brevibacterium* spp., *Listeria* spp., *Azotobacter* spp., *Cellulomonas* spp., *Corynebacterium* spp., *Terrabacter* spp., *Aerococcus* spp., *Klebsiella* spp., *Marinococcus* spp., *Sacchrococcus* spp., *Enterobacter* spp., *Thiobacillus* spp., *Planococcus* spp., *Shigella* spp., *Dermobacter* spp., *Salinococcus* spp and *Deinococcus* spp.(Table-3)

Table1. Number of isolated bacterial colonies present in four season of year (2013-2014)

List of Organisms Name	Summer	Premonsoon	Monsoon	Postmonsoon
1. *Micrococcus* spp	+	+	+	+
2. *Bacillus* spp	+	+	+	+
3. *Acetobacter* spp	-	+	+	-
4. *Pseudomonas* spp	+	+	+	+
5. *Streptococcus* spp	+	+	+	-
6. *Staphylococcus* spp	+	+	+	+
7. *Enterococcus* spp	-	+	-	-
8. *Sulfobacillus* spp	+	-	+	+
9. *Escherichia coli*	+	+	+	+
10. *Aeromonas* spp	+	-	-	+
11. *Brevibacterium* spp	-	+	-	-
12. *Listeria* spp	-	-	+	-
13. *Azotobacter* spp	+	-	-	+
14. *Cellulomonas* spp	-	+	-	-
15. *Corynebacterium* spp	+	+	-	+
16. *Terrabacter* spp	+	+	-	-
17. *Aerococcus* spp	+	+	+	+
18. *Klebsiella* spp	+	+	+	+
19. *Marinococcus* spp	+	-	+	-
20. *Sacchrococcus* spp	+	+	+	+
21. *Enterobacter* spp	+	+	+	+
22. *Thiobacillus* spp	+	-	-	-
23. *Planococcus* spp	-	+	+	+
24. *Shigella* spp	+	-	-	-
25. *Dermobacter* spp	-	-	+	-
26. *Salinococcus* spp	+	-	-	-
27. *Deinococcus* spp	-	-	+	-

+ indicates presence; - indicates absence

Table.2 Morphological Characterisation of isolated bacteria

S. No.	List of Organism Name	Morphology and Nature of the colony	Colour of colony	Size of colony(mm)
1	*Micrococcus* spp	Convex,circular	Yellow	0.5-1.5 mm
2	*Bacillus* spp	Flat and irregular	Whitish	1.2 mm`
3	*Acetobacter* spp	Circular	Pale gray	1-3 mm
4	*Pseudomonas aeruoginosa*	Round	Diffusible Green	1.5-3 mm
5	*Streptococcus* spp	Oval,translucent	White	0.5-0.8 mm
6	*Staphylococcus* spp	Convex,circular	Yellow	1-1.8mm
7	*Enterococcus* spp	Regular	Yellow/white	0.8 mm
8	*Sulfobacillus* spp	Oval	White	0.5-1 mm
9	*Escherichia coli*	Round	Cream white	0.5-1.0 mm
10	*Aeromonas* spp	Convex,opaque	Brown	0.8 mm
11	*Brevibacterium* spp	Convex,opaque	Gray-white	2 mm
12	*Listeria* spp	Round,translucent	White	1-2 mm
13	*Azotobacter* spp	Oval	White	1-2 mm
14	*Cellulomonas* spp	Regular	Yellow/white	1-2 mm
15	*Corynebacterium* spp	Opaque,translucent	Purple	0.5-1 mm
16	*Terrabacter* spp	Round	Gray-white to yellow	1-2 mm
17	*Aerococcus* spp	Irregular	Dull white	0.5-1 mm
18	*Klebsilla* spp	Round	Grayish White	1-2 mm
19	*Marinococcus* spp	Irregular	Yellow/orange	0.1-0.5 mm
20	*Sacchrococcus* spp	Irregular	White	0.5-1.5 mm
21	*Enterobacter aerogenes*	Convex	Yellow	0.5-1mm
22	*Thiobacillus* spp	circular	White	1-3 mm
23	*Planococcus* spp	circular	Orange	1-2 mm
24	*Shigella* spp	Circular,translucent	White	0.5-1 mm
25	*Dermobacter* spp	Circular	Greyish white	0.5-1 mm
26	*Salinococcus* spp	Circular	Orange	0.8-1.2 mm
27	*Deinococcus* spp	Regular	Red to pink	0.3-1.5 mm

Table.3 Bio chemical Characterization

S. No	List of Organisms	Gram Staining	Motility	Indole	MR	VP	Citrate	Catalase	Urease	TSI	Oxidase	Nitrate Reduction Test	Carbohydrates Fermentation Test		
													G	L	S
1.	***Micrococcus* spp**	+ve cocci	Non-Motile	+	-	+	-	+	+	Alkaline production	-	-	-	-	+
2.	***Bacillus* spp**	+ve Rod	Motile	-	+	+	+	+	-	Alkaline,Gas production	+	-	+	+	
3.	***Acetobacter* spp**	-ve Rod	Non-Motile	-	-	-	-	+	-	Acid production	-	+	+	-	-
4.	***Pseudomonas aeruoginosa***	-ve Rod	Motile	-	-	-	+	+	-	No change of production	+	+	+	-	-
5.	***Streptococcus* spp**	+ve cocci	Non-Motile	-	-	-	+	-	-	Alkaline production	-	-	+	+	+
6.	***Staphylococcus* spp**	+ve cocci	Non-Motile	-	-	-	+	+	+	Alkaline production	-	-	+	+	+
7.	***Enterococcus* spp**	+ve cocci	Non-Motile	-	-	+	-	-	-	No Acid,Gas production	-	-	+	+	+
8.	***Sulfobacillus* spp**	+ve Rod	Non-Motile	-	-	-	+	+	-	Alkaline production	+	-	+	+	+
9.	***Escherichia coli***	-ve Rod	Motile	-	-	+	-	-	-	Acid,Gas production	-	+	+	+	+
10.	***Aeromonas* spp**	-ve Rod	Motile	+	-	+	+	+	-	No acid production	+	-	+	-	+
11.	***Brevibacterium* spp**	+ve Rod	Non-Motile	-	+	-	+	+	-	Alkaline production	+	-	+	-	+
12.	***Listeria* spp**	+ve Rod	Motile	-	+	+	-	+	-	Acid production	-	-	-	-	-
13.	***Azotobacter* spp**	+ve Rod	Non-Motile	-	+	-	+	+	+	Alkaline, Acid production	+	-	+	+	+
14.	***Cellulomonas* spp**	+ve Rod	Motile	-	-	-	-	+	-	No change of production	-	+	+	+	+
15.	***Corynebacterium* spp**	+ve Rod	Non-Motile	-	+	-	-	+	+	Acid production	-	+	+	+	-
16.	***Terrabacter* spp**	+ve Rod	Non-Motile	-	-	-	-	-	-	No acid production	+	+	-	-	-
17.	***Aerococcus* spp**	+ve cocci	Non-Motile	+	+	-	+	+	+	Alkaline production	+	-	+	+	+
18.	***Klebsiella* spp**	-ve Rod	Non-Motile	-	-	+	+	+	+	Acid,Gas production	-	-	+	+	+
19.	***Marinococcus* spp**	+ve cocci	Motile	-	-	-	+	+	-	Alkaline production	+	+	-	+	+
20.	***Sacchrococcus* spp**	+ve cocci	Non-Motile	-	+	-	+	+	+	Alkaline production	+	-	+	+	+
21.	***Enterobacter* spp**	-ve Rod	Motile	-	-	+	+	+	+	Gas production	-	+	+	+	-
22.	***Thiobacillus* spp**	-ve Rod	Non-Motile	-	-	+	-	+	-	Acis,Gas production	+	-	+	+	+

23.	***Planococcus* spp**	+ve cocci	Non-Motile	+	+	-	+	+	-	Alkaline production	-	-	+	+	-
24.	***Shigella* spp**	-ve Rod	Non-Motile	-	+	-	-	+	-	H2S not produced	-	-	-	-	-
25.	***Dermobacter* spp**	+ve Rod	Non-Motile	-	-	-	+	+	-	Acid, Alkaline production	+	-	+	+	+
26.	***Salinococcus* spp**	+ve rod	Motile	-	-	-	-	+	-	H2S production	+	+	+	-	-
27.	***Deinococcus* spp**	+ve Rod	Non-Motile	+	-	-	+	+	-	Alkaline production	+	-	+	+	+

Several studies suggested that soil microbial diversity had seasonal fluctuations (Lipson and Schmidt, 2004; Smit *et al*., 1997). Presence or absence of particular bacterial genera may depend on soil parameters, as observed by Alexander (Alexander, 1971). The Gram negative population and higher O_2 level in sea water is conformed to previous reports by Gonzalez-Acosta and Ascencio *et al.* (2006).

Conversely the present study exposed higher Gram positive bacteria. The site of the present study soil supports for the presence of higher population of gram positive compare with gram negative, nitrifier, denitrifiers, phosphate solubilizer, sulphur oxidizers, for major biogeo-chemical cycles and also bioluminescent bacteria responsible for quorum sensing.

In conclusion, Mangrove soils endow with sanctuary and nurturing sites for many marine bacteria. Conservation strategies for mangroves should consider the ecosystem as a biological entity, which includes all the physical, chemical, and ecological processes that maintain productive mangroves. Outstanding to the presence of wealthy source of nutrients mangroves are called the homeland of microbes. Extensive exploration, identification, isolation and screening are suggested in search of new leads for microbial drugs.

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