Original Research Article

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

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ABSTRACT

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

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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.
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., Thioecillus 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. Thioecillus 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 aeruginosa| Round                               | Diffusible Green | 1.5-3 mm           |
| 5      | Streptococcus spp     | Oval, translucent                   | White            | 0.5-0.8 mm         |
| 6      | Staphylococcus spp    | Convex, circular                    | Yellow           | 1-1.8 mm           |
| 7      | Enterococcus spp      | Regular                             | Yellow/white     | 0.8 mm             |
| 8      | Sulfabacillus 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     | Sacbroccocus spp      | Irregular                           | White            | 0.5-1.5 mm         |
| 21     | Enterobacter aerogenes| Convex                             | Yellow           | 0.5-1 mm           |
| 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: Biochemical Characterization

| S. No | List of Organisms     | Gram Staining | Motility | Indole | MR | VP | Citrate | Catalase | Urease | TSI | Oxidase | Nitrate Reduction Test | Carbohydrates Fermentation Test | Carbohydrates Test |
|-------|-----------------------|---------------|----------|--------|----|----|---------|----------|--------|-----|---------|------------------------|---------------------|------------------|
| 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* aeruginosa | -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.   | *Saccharococcus* 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 | +                   | +                   | +                |
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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