

Phyllosphere Microbial Populations of Ten True Mangrove Species of the Andaman Island

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Abstract: Bacterial, fungal and actinobacterial populations in ten true mangrove species of the Andaman Island were studied to evaluate differences in their occurrence associated with the host species. It was found that at uniform salinity, bacterial, fungal and actinobacterial populations occurred with different density in the mangrove phyllosphere. *Rhizophora* spp showed higher fungal population and *Bruguiera gymnorhiza* and *Ceriops tagal* exhibited high bacterial population. Actinobacterial population was higher in *B. gymnorhiza*. There was complete absence of bacterial population in *B. cylindrica* and fungal population in *C. tagal*, *Heritiera littoralis* and *Schyphiphora hydrophyllacea*. In similarly pore water salinity, fungal population decreased from frontline members (proximal zone) to backward mangroves (distal zone). Bacterial population was higher in the members of middle zone, whereas those of the proximal and distal zones showed similar bacterial population density. Actinobacterial population was also higher in the middle and lower in the proximal and distal zones. Occurrence of only two combinations of microbial population (either bacteria and fungi or bacteria and actinobacteria) in most of the true mangrove species indicates that biological competition might exist among the different groups of microbial communities occurring in the phyllosphere of mangrove plants and this aspect is worth further pursuit.

Key words: Mangrove • Phyllosphere • Bacteria • Fungi • Actinobacteria andaman Island

INTRODUCTION

Mangroves are the salt-tolerant forest ecosystems that occur mainly in the tropical and sub-tropical intertidal regions of the world. In the Andaman district, the area under mangrove cover is 612 km², while in Nicobar district mangrove cover is only 3 km² (FSI, 2009). As many as 34 exclusive species distributed among 17 genera and 13 families are reported from Andaman and Nicobar (ANI) [1]. Mangroves occurring in these islands are mostly fringing the creeks, backwaters and muddy shores [2].

Ecologically microorganisms play an important role in decomposing organic matter and producing protein-rich detritus that serves as food to fishes especially in detritus-based marine ecosystems like mangroves [3- 5]. Microbial diversity of mangrove soil and microbial decomposition of mangrove litter has been extensively

studied [6-17] especially for the prevalence of fungi in decaying seedlings [18]. But the microbial flora of phyllosphere of mangroves and their ecological significance is not yet explored.

The phyllosphere is the living leaf as a whole and includes the interior and surface [19] and is colonized by a variety of microorganisms [20, 21]. The phyllosphere microbial flora is of a special interest from various points of view because some of them have antagonistic action against fungal parasites, degrade plant surface wax and cuticles, activate the host plant to produce phytoalexins, act as a source of allergic air borne spores and influence growth behavior and root exudation of plants [22]. So, the present work was focused to assess the population of bacteria, fungi and actinobacteria associated with the phyllosphere of ten true mangrove species of the Andaman Island.

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MATERIALS AND METHODS

Mangroves of Barmanallah, Port Blair (Andaman) occupy a littoral habitat, characterized almost invariably by salt or brackish water and coastal silt exposed to daily tidal inundation with a continuously changing salinity and represented by tree mangroves from the genera *Avicennia*, *Aegiceras*, *Bruguiera*, *Ceriops*, *Excoecaria*, *Heritiera*, *Rhizophora*, *Sonneratia* and *Scyphipora*. Mangroves of Barmanallah are fringing type, present on both the banks of the tidal creek. Phyllosphere samples were collected from ten true mangrove species zone wise: *Rhizophora apiculata* (Blume) and *R. mucronata* (Poir) represent the proximal zone, *Aegiceras corniculatum* [(L.) Blanco], *Bruguiera gymnorhiza* [(L.) Lamk], *B. cylindrica* [(L.) Bl.], *B. parviflora* (Wight and Arnold ex Griffith) and *C. tagal* [(Perr.) C.B. Robinson] represent the middle zone, *Heritiera littoralis* (Dryand. In Aiton), *Xylocarpus granatum* (Koenig) and *Scyphipora hydrophyllacea* (Gaertn. f) represent the distal zone. Leaf samples of each species were collected. Prior to sample collection, pore water salinity of rhizosphere of each mangrove species was determined using refractometer. In addition to it ten leaves of each species were collected for analysis of water content.

The leaf samples were thoroughly washed with sterile distilled water prior to inoculation. The surface sterilization of leaves was made with 0.1% HgCl₂ for 30 s and again rewashed by flowing sterile distilled water for 10 min. The leaves were cut into 5 mm disc through cork borer. Five numbers of leaf discs of each sample were dipped into 10 ml of sterile distilled water and subjected to serial dilution after vigorous shaking. The 10⁻³ diluted triplicate samples were inoculated on marine agar, potato dextrose agar and actinomycetes isolation agar (Actinomyces agar was supplemented with 80 µg/ml of cycloheximide and 75 µg/ml of nalidixic acid (Himedia, Mumbai) to minimize the other bacterial and fungal growth) and incubated at 37°C and 28°C for 2 and 4 days to procure bacterial and fungal colonies, respectively and 14 days at 30°C for actinobacteria [26]. Bacterial colonies on marine agar (Hi Media) obtained through the serial dilution technique were counted by colony counter. The total population of bacteria, fungi and actinobacteria were calculated and presented in terms of number per square centimeter leaf area. Finally, data were analyzed for variance (one way ANOVA) among and between the bacteria, fungi and actinobacteria [23].

RESULTS

Mangrove plants of the middle zone were found to be populated with high number of bacteria and actinobacteria than in the proximal and distal zone species. Fungal population was higher in the mangrove plants of the studied proximal zone. Among the ten true mangrove species studied, most of the species exhibited higher bacterial population than fungal and actinobacterial populations except *R. apiculata*, *R. mucronata* and *B. cylindrica*. Leaves of *B. gymnorhiza* showed the highest bacterial population density (153.7x10⁻³) followed by *C. tagal* (38.2x10⁻³) and *X. granatum* (30.5x10⁻³). All the other species except *B. cylindrical* showed almost a similar range of bacterial population, ranging between 4.2 x 10⁻³ to 5.9x10⁻³ (Table 1). It was observed that most of the mangrove species exhibited very low fungal population except *R. apiculata* (82.7x10⁻³), *R. mucronata* (36.5x10⁻³) and *X. granatum* (13.5x10⁻³).

Mangrove species in proximal zone namely *R. apiculata* and *R. mucronata* exhibited almost similar bacterial population. Actinobacterial population occurred only in *R. apiculata* (9.34X10³), which was greater than the bacterial population (5.9X10³) and less than the fungal population (82.7X10³).

Among the 5 species of the middle zone, *B. gymnorhiza* exhibited higher bacterial population (153.7X10³) followed by *C.s tagal* (38.2X10³). However, in both the mangrove species, fungal population was absent. In *B. cylindrical* bacterial population was absent but it showed fungal population. Both *A. corniculatum* and *B. parviflora* exhibited uniform bacterial and fungal populations. Actinobacterial population was found in *B. gymnorhiza* and *C. tagal* (Table-1).

Among the three distal zone species, *X. granatum* exhibited the highest bacterial population (30.5X10³) and the fungal population was found only in *X. granatum*. Actinobacterial population was found only in *H. littoralis*.

It was observed that except *B. cylindrical*, *S. hydrophyllacea* and *R. apiculata*, phyllosphere of all these mangrove species was populated only by any two populations (either bacteria and fungi or bacteria and actinobacteria). In addition none of the mangrove phyllosphere was populated with equal density of the microbes. Only, *R. apiculata* exhibited all the three microbial populations. *S. hydrophyllaceae* showed only bacterial population, whereas *B. cylindrical* showed only fungal population.

Table 1: Microbial population per cm² of phyllosphere (mean and SD) of ten true mangroves species of Barmanallah (Andaman)

| Zone | Name of the species | Bacteria x10 ⁻³ | | Fungi x 10 ⁻³ | | Actinomyces x 10 ⁻³ | | Leaf water content (%) | Pore water Salinity (PPT) |
|---------------|--------------------------------------|----------------------------|------|--------------------------|------|--------------------------------|------|-------------------------|---------------------------|
| | | Mean | SD | Mean | SD | Mean | SD | | |
| Proximal Zone | <i>Rhizophora apiculata</i> | 5.9 | 3.1 | 82.7 | 23.9 | 9.34 | 0.57 | 68.1 | 25 |
| | <i>Rhizophora mucronata</i> | 4.2 | 1.2 | 36.5 | 12.7 | 0 | 0 | 71.0 | 25 |
| Middle Zone | <i>Aegiceras corniculatum</i> | 5.1 | 2.0 | 5.9 | 1.4 | 0 | 0 | 59.0 | 25 |
| | <i>Bruguiera gymnorhiza</i> * | 153.7 | 46.1 | 0 | 0 | 45.01 | 1.47 | 61.5 | 25 |
| | <i>Bruguiera cylindrica</i> * | 0 | 0 | 4.2 | 2.4 | 0 | 0 | 68.3 | 24 |
| | <i>Bruguiera parviflora</i> | 4.2 | 2.4 | 4.6 | 2.6 | 0 | 0 | 61.6 | 24 |
| | <i>Ceriops tagal</i> * | 38.2 | 9.5 | 0 | 0 | 5.09 | 2.55 | 62.6 | 25 |
| Distal Zone | <i>Heritiera littoralis</i> * | 4.2 | 1.2 | 0 | 0 | 1.66 | 1.54 | 59.1 | 25 |
| | <i>Scyphiphora hydrophyllaceae</i> * | 5.9 | 1.2 | 0 | 0 | 0 | 0 | 76.1 | 24 |
| | <i>Xylocarpus granatum</i> | 30.5 | 8.3 | 13.5 | 3.1 | 0 | 0 | 71.4 | 24 |

Although mangroves species were selected zone wise, uniform pore water salinity and leaf water content suggests that in the study area, all the mangroves species experienced almost similar salinity (Table-1). Fungal population was low, comparatively towards the land ward side (distal zone) and bacterial population was higher in the middle zone mangroves. Species of *Rhizophora* showed higher fungal population, species of *Bruguiera* and *Ceriops* exhibited higher bacterial population towards the land ward side (distal zone) where fungal population in phyllosphere was completely eliminated, of mangroves like *S. hydrophyllaceae* and *H. littoralis* and frontline members (proximal) like *R. apiculata* and *R. mucronata* showed lower bacterial population.

DISCUSSION

Difference in the occurrence of bacteria, fungi and actinobacteria in the phyllosphere of mangroves of different zones has indicated that marine microbes have a significant role to play in local mangrove communities [24]. Members of middle and distal zone species can withstand the salinity by salt excretion through leaves (e.g. *A. corniculatum*). So the poor colonization of fungi on mangrove plants of middle and distal zones might be due to salt excretion in leaves, which serves as an important defense mechanisms against fungal attack and /or colonization [25]. Previously it has been reported that bacterial and fungal populations are higher in low salinity zone [26], present study probably due to as against finding of the lack of distinct zonation pattern in the mangroves of the Andaman and Nicobar Islands (ANI). Most of the mangrove areas in the Andaman and Nicobar Islands has nearly less than 1km [27]. So, the backward members might also experience the same salinity as frontline members.

It is quite apparent from the present study that bacterial, fungal and actinobacteria populations did not occur in similar density on the mangrove species. At uniform salinity, fungal population was higher in proximal zone members and got eliminated towards the distal zone. Bacterial and actinobacterial populations were higher in the members of the middle zone than the proximal and distal zone members. Compared to the studies on microbial diversity in different ecosystem, research on the microbial features of in the mangroves is in its infancy; particularly studies on phyllosphere microbiology are very limited. In this context, information elucidated in the present study would help pursue microbial research for different objectives in the mangrove areas of the Andaman Islands.

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