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Microbial flora associated with submerged mangrove leaf litter in India

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Abstract: We studied the microbial flora in decomposing mangrove leaves in relation to changes in nitrogen and tannin levels, and in penaeid prawn assemblages. Senescent leaves of two mangrove species (*Rhizophora apiculata* and *Avicennia marina*) kept in nylon bags, were separately immersed for 80 days in five tanks full of mangrove water. A known amount of decomposing leaves was collected every ten days from each tank for microorganism counts, total nitrogen and tannin measurement, and juvenile penaeid prawn counts. Five genera of total heterotrophic bacteria (THB), three species of azotobacters and 19 species of fungi were identified. The azotobacters showed a significant peak around 40-50 days after the beginning of of decomposition, similar to the trend for total nitrogen and for prawn assemblages. Rev. Biol. Trop. 55 (2): 393-400. Epub 2007 June, 29.

Key words: *Rhizophora, Avicennia,* litter, microbes, decomposition, *Azotobacter*.

Microorganisms play an important ecological role in decomposing organic matter and producing protein-rich detritus that serves as food to fishes especially in detritus-based marine ecosystems like mangroves (Dickinson and Pugh 1974, Steinke 2000, Mumby *et al*. 2004). Fungi are particularly important in the marine environment as decomposers of dead organic substrates (Kohlmeyer and Kohlmeyer 1979). The dead organic matter and the associated microorganisms form the base of the food webs of commercially important fishes and crustaceans. The undecomposed leaves are poor in nutrients, and they become nutritious due to the microbial enrichment process during decomposition (Odum 1971). The microbial decomposition of mangrove litter, has been extensively studied (Fell and Master 1973, 1980, Meyers 1974, Fell *et al.* 1975, 1980, Boonruang 1978, Cundell *et al*. 1979, Misra *et al*. 1984, Robertson 1988, Raghukumar *et al.* 1994, Rajendran and Kathiresan 1999a, 2000, Kathiresan and Bingham 2001, Rajendran and Kathiresan 2004) especially for the prevalence of fungi in decaying seedlings (Newell 1976). The microbial activity results in mineralization of detritus and a decrease in C/N ratio (Blum *et al.* 1988).

Although microbes play an important role in the cycling of nutrients in the mangrove ecosystem, very little information is available about the types of microbes associated with decomposing leaves. These studies have not been extended to faunal assemblages associated with the decomposing leaves, especially for juvenile penaeid prawns which are usually abundant in mangrove waters (Rajendran 1997, Rajendran and Kathiresan 1999b, 2004). Hence, the present study was made on microbial load during decomposition of two mangrove species.

MATERIALS AND METHODS

Senescent leaves of *Avicennia marina* (Forsk.) Vierh., 1907 and *Rhizophora apiculata* Blume, 1827 were collected from a

mangrove forest at Pichavaram (11°27' N, 79°47' E) located in southeast coast of India and shade-dried. The leaves weighing at 500 g, without dissection, were packed in five nylon bags (35 x 35 cm, 2 mm mesh size) and submerged in five tanks (1.5 x 1.0 m), which were constructed separately for *Rhizophora* and *Avicennia* in the lower intertidal areas adjacent to the mangrove forest. To facilitate the bags to sink, a stone weighing at 0.5 kg for each bag was added. The inner tank was covered with 2 mm (mesh size) nylon net to trap the juvenile prawns during decomposition of leaves. This experiment was continued for 80 days in the pre-monsoon months (July-September 1999). During this period, the water temperature fluctuated between 27 °C and 32 °C. The pH remained between 7.6 and 8, the salinity varied between 24 and 31gl^{-1}, and the dissolved oxygen ranged from 3.2 to 5.6 ml l^{-1}. At every ten days of analysis, a known amount of decomposing leaves was collected randomly from each litter bags of both species for microbial and chemical analysis.

Level of total nitrogen in the decomposing leaves was analyzed using Technicon Autoanalyzer (Gradko Industrial Ltd., UK) and also level of tannins using the method described by Kathiresan and Veera Ravi (1990). During decomposition, prawn jóvenes were collected from the tank by lifting the inner net and were identified using the key characters given by Paulpandian and Ramasamy (1991).

For microbial analysis, the decomposing leaves were collected in clean and sterile polybags from litter bags and aseptically cut in to small pieces. These were then washed with sterilized seawater to remove debris on the leaves. Then they were dipped in 0.01 % $HgCl_2$ solution for 3 min for surface-sterilization of the pieces. The pieces were then washed with sterilized seawater to remove all the traces of $HgCl_2$ solution. For estimating fungi, the pieces were then placed on plates with Martin rose bengal agar (Hi-Media Laboratories, Mumbai, India) medium incorporated with an antibiotic mixture (chlorotetracycline-HCl 10 %, chloramphenicol 2 % and streptomycin sulphate 2 %; Van Uden and Fell 1968) for suppressing bacterial growth in the media.

For estimating total heterotrophic bacteria, ZoBell 2216E agar (Hi-Media Laboratories, Mumbai, India) medium was used; and for azotobacters, nitrogen-free mannitol agar medium was used (Buchanan and Gibbons 1974). Triplicate plates were incubated at room temperature (28±2 °C) for 2-7 days and the colonies were counted. The microbial counts are expressed as the number of colony forming units (CFU) per gram of wet leaf tissue. Bacteria including azotobacters were identified referring to Bergey's manual (Buchanan and Gibbons 1974) and fungi were identified following the keys given by Ainsworth *et al*. (1973) and Raper and Fennell (1987). The values were treated with two way analysis of variance (ANOVA) to find out the significance between the microbial load and other parameters. Correlation analysis between the variables was also analyzed (Snedecor 1956).

RESULTS

Total heterotrophic bacteria: In general, the total heterotrophic bacterial (THB) counts were more in decomposed leaves than in undecomposed ones. The counts reached a peak between 30 and 40 days of decomposition and declined thereafter. In *Rhizophora apiculata*, bacterial counts ranged from 1.60 x 10^5 to 5.58 x 10^5 (no. g wet $tissue^{-1}$) in the leaves decomposed for 10 and 30 days respectively as against 1.10 x 10^5 in undecomposed leaves. In *Avicennia marina*, the counts ranged from 1.78 x 10^5 to 8.56 x 10^5 in the leaves decomposed for10 and 40 days respectively, as against 1.36 x 10^5 in the undecomposed leaves. However, there was no statistically significant variation in THB count between mangrove species (ANOVA= 0.91, df= 1) and also among the days of decomposition (ANOVA= 2.16, df= 8). The bacteria identified at generic levels were *Flavobacterium,*

Vibrio, Pseudomonas, Acinetobacter and *Corynebacterium.*

Azotobacters: In general, count of azotobacters was more in decomposing leaves than in undecomposed ones (Fig. 1) and its peak existed between 30 and 50 days of decomposition and declined thereafter. This trend was similar in both species of mangroves. In *R. apiculata*, the counts ranged from 1.16 x 10^4 to 6.82 x 10^4 (no. g wet tissue^{-1}) in the leaves decomposed for 10 and 50 days respectively as against 1.33 x 10^4 in the undecomposed ones. In *A. marina*, the counts varied from 1.5 x 10^4 to 7.7 x 10^4 in the leaves decomposed for 10 and 40 days respectively, as against 2.6 x 10^4 in the undecomposed leaves. Of the two species of mangroves, the average count was more in *A. marina* (4.11 x 10^4) than in *R. apiculata* (3.20 x 10^4). There was a statistically significant variation in counts of azotobacters between the days of decomposition (ANOVA= 10.03, df= 8, $p<0.01$), but not between the mangrove species (ANOVA= 2.47, df= 1). Three species of azotobacters viz., *A. chroococcum, A. vinelandii* and *A. beijerinckii* were identified. The azotobacters exhibited a significant positive correlation ($r= 0.785$, $p<0.05$) with nitrogen in decomposing leaves.

Fungi: In general, the fungal counts attained a peak between 20 and 30 days of decomposition and declined thereafter. In *R. apiculata*, the counts were recorded maximum of 4 464 (no. g wet tissue^{-1}) in the 30 day decomposed leaves and minimum of 255 (no. g wet tissue^{-1}) in the 80 day decomposed leaves, as against 500 (no. g wet tissue^{-1}) in the undecomposed leaves. In *A. marina*, the fungal counts were recorded maximum of 1 810 (no. g wet tissue^{-1}) in the 20 day decomposed leaves and minimum of 142 (no. g wet tissue^{-1}) in the 80 day decomposed leaves as against 700 (no. g wet tissue^{-1}) in the undecomposed leaves. However, there was no statistically significant variation in the fungal counts between the mangrove species (ANOVA= 0.29, df= 1) and also among the days of decomposition (ANOVA= 2.02, df= 8).

The predominant fungal species identified from decomposing mangrove leaves were belonging to the genus *Aspergillus*. The species were *Aspergillus niger, A. glaucus, A. fumigatus, A. candidus, A. verrucosa, A. smithii, A. flavus, A. ochraceus, A. aureolus* and *A. chevalier*. Other species recorded were *Alternaria alternata, Halosarphia fibrosa, Ophiobolus littoralis, Spathulospora lanata, Pontoporeia biturbinata, Fusarium* sp.*, Mucor* sp., *Penicillium* sp. and *Curvularia* sp.

Total Nitrogen: Total nitrogen was 0.11 % in *R. apiculata* and 0.17 % in *A.marina* on the initial day of decomposition (Fig. 2). The level increased on the 30th day onwards and showed maximum levels of 0.34 % in the 50 day decomposed leaves of *R. apiculata* and 0.32 % in the 40 day decomposed leaves of *A. marina*. There was a statistically significant variation in leaf nitrogen among the days of decomposition

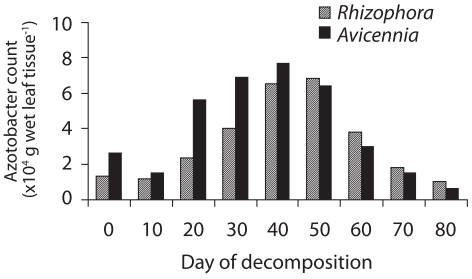

Fig. 1. Azotobacters in decomposing mangrove leaves.

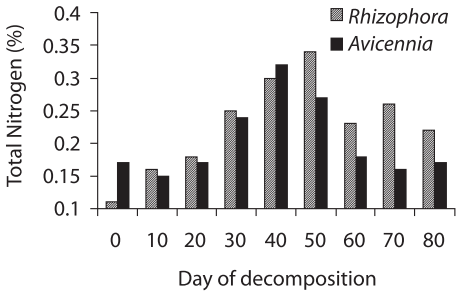

Fig. 2.Total nitrogen content in decomposing mangrove leaves.

(ANOVA= 6.24, df= 8, $p<0.01$), but not between the mangrove species (ANOVA= 2.28, df= 1).

Tannin: The content of tannin in decomposing mangrove leaves is shown in Fig. 3. The tannin content decreased during leaf decomposition, from 36.7 mg g^{-1} on the initial day to 1.9 mg g^{-1} on the 80 days of decomposition in *R. apiculata* and similarly from 18.3 mg g^{-1} on initial day to 1.9 mg g^{-1} on 80 days of decomposition in *A.marina*. Thus there was 94.7 % loss of tannin in *R. apiculata* and 89.6 % in *A. marina* within 80 days of decomposition. There was a significant difference in the content of tannin between the days of decomposition (ANOVA = 5.93, df = 8, $p<0.05$), and also between the mangrove species (ANOVA = 5.75, df = 1, $p<0.05$).

Juvenile prawns: The juvenile prawn's assemblage around decomposing mangrove leaves is depicted in Fig. 4. A higher number of juvenile prawns were found associated with decomposing *A. marina* leaves than *R. apiculata.* The number was 5 $tank^{-1}day^{-1}$ in the former and 2 $tank^{-1}day^{-1}$ in the later in 40 days decomposed leaves. The values were statistically significant between species (ANOVA= 5.22, df= 1, $p<0.05$) and among days of decomposition (ANOVA= 6.06, df= 8, $p<0.01$). The dominant prawn species identified were *Penaeus indicus*, *Metapenaeus monoceros*, *M. dobsoni*, *M. brevicornis* and *Macrobrachium* sp. The juvenile prawns exhibited a significant positive correlation (r= 0.909, $p<0.01$) with nitrogen in decomposing leaves.

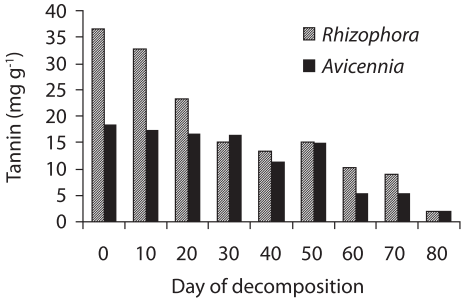

Fig. 3. Content of tannin in the decomposing mangrove leaves.

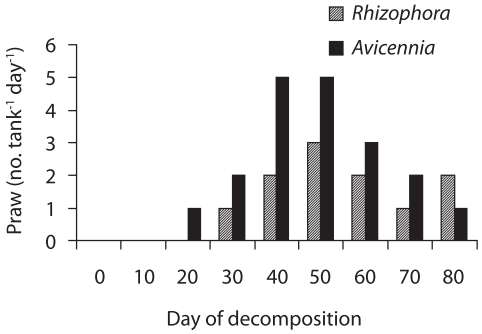

Fig. 4. Juvenile prawns associated with decomposing mangrove leaves.

DISCUSSION

The microbial counts increased with days of decomposition of mangrove leaves. However, only the azotobacters showed a statistically significant increase during leaf decomposition. The azotobacters, isolated from the decomposed mangroves, were already proved in this laboratory to fix nitrogen (Ravikumar 1995). The nitrogen content, which was rich in decomposing leaves, as was found in the present study (Fig. 2), could be at least partially due to nitrogen-fixing bacteria like azotobacters (Fig. 1). Besides microbial biomass, it is now believed that increase in nitrogen content of marine detritus is due to the complexing of microbial exudates and enzymes with phenolics and carbohydrates to form recalcitrant, humic nitrogen (Melillo *et al*. 1984, Raghukumar *et al*. 1995).

The azotobacters which enrich nitrogen in the decomposing mangrove leaves may be a major factor in determining the palatability of detritus food to detritivorous animals like prawns. This supports our observation that there was an increasing trend in assemblage of juvenile prawns with days of leaf decomposition, with a peak around 40-50 days (Fig. 4), which was similar to the trend of total nitrogen content (Fig. 2) and azotobacter counts (Fig. 1).

There was a highly significant positive correlation between

The count of azotobacters and nitrogen content of decomposing leaves ($r= 0.785$, $p<0.05$) and between nitrogen and juvenile prawns, associated with decomposing mangrove leaves ($r= 0.909$, $p<0.01$). Thus the azotobacters increase the level of nitrogen in decomposing mangrove leaves which perhaps attracts juvenile prawns.

The bacterial assemblage appears to depend on the content of tannin in the decomposing leaves. In the initial days of decomposition, the tannin content was high coinciding with low bacterial count. After the gradual leaching of tannin, the counts also increased. But the fungal count was high when content of tannin was high in the initial days of decomposition, after that the count declined with lowering of tannin (Rajendran 1997, Rajendran and Kathiresan 2000). This early colonization of fungi in tannin-rich mangrove leaves may be due to the fact that the fungi like Aspergilli are capable of producing tannase enzyme to degrade tannin (William *et al.* 1986). Tannin is known to inhibit the growth of fungi, but stimulate at low concentration (De *et al*. 1982). The bacterial diversity was high when phenol concentration was low in the mangrove sediment and the rate of biodegradation of leaves reduced at the high concentration of phenol (Joseph and Chandrika 1999, 2000).

In the present work, 19 species of fungi were isolated from the decomposing mangrove leaves with predominance of *Aspergillus* spp. Most of the species encountered in the present study were terrestrial forms, as was also observed by other workers (Rai *et al.* 1969, Venkatesan 1981, Miyoshi *et al*. 1985, Mohamed Salique 1989). The reason might be attributed to large scale transport of fungal spores from the land through freshwater inflow in to mangrove ecosystem. Several workers have already isolated mostly terrestrial species from the study area. Periasamy (1979) isolated 33 species of fungi from rhizosphere soil, pneumatophores and leaf litter of *Avicennia officinalis*. Venkatesan (1981) isolated 35 species of cellulolytic fungi from soil, root and litter of *Avicennia officinalis* and *Rhizophora mucronata*. Mohamed Salique (1989) recorded 23 species of fungi from the litter of *R. apiculata* and 19 species from *Bruguiera cylindrica*. The same author recorded 14 species of *Aspergillus*, with predominance of *A. fumigatus* and *A. niger*. Sivakumar and Kathiresan (1990) reported 10 species of fungi, found to occur on the surface of mangrove leaves, with dominance of *Alternaria alternata* and *Rhizopus ingricam* followed by *Aspergillus* and *Penicillium* species. Ravikumar and Kathiresan (1993) reported higher number of fungi on leaf litter than those on fresh leaves. Thirty one fungal isolates were recorded from soil and 27 species from decaying mangroves and seven species from floating plants were reported with dominance of *Aspergillus* followed by *Penicillium*, *Fusarium* and *Trichoderma* in Mangalvan mangrove ecosystem (Prabhakaran *et al.* 1987). Sixty seven fungal species were recorded from the intertidal wood samples with dominance of *Lulworthia* species and forty eight fungal species were identified from dead parts of *Rhizophora mucronata* prop roots (Poonyth *et al.* 1999). Sixty one species of higher marine fungi were collected from the submerged wood blocks of *Bruguiera gymnorhiza* and *Rhizophora mucronata* in Mauritius water (Poonyth *et al.* 2001). Seventy three species of fungi from Godavari and 67 species from Krishna estuaries of India were collected from the decaying samples of *Rhizophora* and *Avicennia* (Venkateswara Sarma *et al*. 2001). A total of 78 species of fungi belonging to 45 genera comprising 46 ascomycetes, one basidiomycetes and 31 deuteromycetes were recorded from the dead woods of mangroves in different parts of India (Maria and Sridhar 2002). One hundred and twenty five strains of endophytic fungi were isolated from the inner barks of three kinds of mangrove plants (Zheng 2003).

Both fungi and bacteria are important decomposers of mangrove leaves (Mohamed Salique 1989, Rajendran 1997). The role of fungi in decomposition of *Rhizophora apiculata* has also been proved under laboratory (Raghukumar *et al*. 1994). However, in the

present study, there were no significant fungal and bacterial densities among the days of leaf decomposition, except for azotobacters. There might be significant variation in the microbial activities especially of enzymatic breakdown of decomposing leaves, which deserve more attention.

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RESUMEN

Se estudió la flora microbiana en hojas en descomposición de mangles, considerando nitrógeno, taninos y camarones peneidos jóvenes. Colocamos hojas viejas de dos especies de mangle (*Rhizophora apiculata* y *Avicennia marina*) en bolsas de nylon y las sumergimos en agua de manglar durante 80 días usando cinco tanques separados. Cada diez días extrajimos una cantidad conocida de hojas en descomposición de cada tanque. Hallamos cinco géneros de bacterias heterotróficas totales (THB), tres especies de azotobacterias y 19 especies de hongos. Las azotobacterias presentaron un pico significativo de abundancia alrededor de los 40-50 días de descomposición, un patrón similar a los del nitrógeno total y los camarones.

Palabras clave: *Rhizophora, Avicennia,* hojarasca, microorganismos, descomposición, *Azotobacter*.

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