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MICROBIAL POPULATION IN PHYLLOSPHERE OF MANGROVES GROW IN DIFFERENT SALINITY ZONES OF BHITARKANIKA (INDIA)

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SUMMARY. *Microbial population in phyllosphere of mangroves grow in different salinity zones of Bhitarkanika (India)*. The bacterial and fungal populations in phyllosphere of mangrove plants were investigated in order to evaluate differences in their occurrence associated with host species. Study sites included relatively undisturbed and purely mangrove area that were selected for sampling from both the low and intermediate salinity zones. Microbial population count was analyzed in 11 and 14 different and/or similar plant species from these two salinity zones. The maximum microbial population was observed in phyllosphere of low salinity zone. However, *Crinum defixum* L. was found to be most populated with bacteria among all other phyllosphere plant samples tested.

Key words. Mangrove, bacteria, fungi, salinity, Bhitarkanika.

RESUMEN. *Poblaciones microbianas de las hojas de manglares que crecen en zonas de diferente salinidad de Bhitarkanika (India)*. Se estudiaron las poblaciones de bacterias y hongos que se desarrollan sobre las hojas de manglares con el objeto de evaluar si había diferencias entre las especies hospedadoras. El estudio incluía manglares de áreas no alteradas, y se escogieron zonas que representasen valores de salinidad bajo (11 especies) y media (14 especies), respectivamente. El valor más alto de las poblaciones microbianas se detectó en hojas de manglares de la zona de baja salinidad, pero en la zona de salinidad media *Crinum defixum* L. fue la especie hospedadora con los niveles más altos de poblaciones bacterianas.

Palabras clave. Manglares, bacterias, hongos, salinidad, Bhitarkanika.

INTRODUCTION

Mangrove ecosystems are situated at the inter-phase between marine and terrestrial environment which is highly productive providing nutrients to surrounding microbiota (Alongi, 2005). In response, microbial system participate in biomineralization of organic

matter and biotransformation of minerals. Several foliar fungi and bacteria are reported for their occurrence and beneficial activity in mangrove ecosystem (Ananda & Sridhar, 2004; Kathiresan & Selvam, 2006). Bhitarkanika is second largest mangrove ecosystem in India. A survey has been made towards the population status of bacteria and fungi associated with the phyllopsphere of different mangrove plants.

MATERIALS AND METHODS

Mangroves of Bhitarkanika, Orissa (20°30'-20°50'N, 86°45'-87º10'E) 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*, *Kandelia*, *Rhizophora* and *Sonneratia*. However, there is no available data on the status of microbial population associated with phyllosphere of tree mangroves of Bhitarkanika.

The sampling zone was such a habitat where high spring tides inundate occasionally. The soil reaction changes from alkaline to moderately acidic condition and the zone is mainly colonized by *Acanthus ilicifolius* L., *Acrostichum aureum* L., *Aegiceras corniculatum* Blanco, *Aglaia cucullata* (Roxb.) Pellegr., *Avicennia officinalis* L., *Bruguiera gymnorrhiza* (L.) Lam., *Bruguiera parviflora* (Roxb.) Wight & Arn. ex Griff., *Brownlowia tersa* (L.) Kosterm., *Dalbergia spinosa* Roxb., *Derris heterophyla* (Wild) Back, *Excoecaria agallocha* L., *Heritiera fomes* Buch.-Ham., *Kandelia candel* (L.) Druce and *Sonneratia caseolaris* (L.) Engl. It was covered four different locations i.e. Bhrahmani river, Khola mouth, Khola and confluence point of Khola and Bhitarkanika.

The mid tidal zone along the creeks is greatly influenced by intermediate salinity and tidal action and largely colonized by *A. ilicifolius*, *A. corniculatum*, *A. cucullata*, *A. officinalis*, *Caesalpinia crista* L., *D. heterophyla*, *E. agallocha*, *H. fomes*, *Sesuvium portulacastrum* (L.) L., *S. caseolaris* and *Tamarix troupii* Hole. It was covered five locations i.e. Balizore, Brahmamari creek, Dangmal, Mahishmari creek and confluence point of Balizore and Khola.

The above mentioned locations were visited through country boat and plant samples were collected in pre-sterilized sample containers. Total 9 and 14 plant samples in triplicate were collected from intermediate and low salinity zones, respectively. It was identified by one of our author (UCB) on the spot and brought to the laboratory for further studies.

The leaf samples were thoroughly washed with sterile distilled water prior to inoculation. The surface sterilization of leaves were made through 0.1% $HgCl_2$ for 30 s and again rewashed through 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 in dipped into 10 ml of sterile distilled water and subjected to serial dilution after vigorous shaking. The 10^{-3} diluted samples were inoculated on marine agar and potato dextrose agar and incubated at 30ºC and 37ºC for the 7 days to procure bacterial and fungal colonies (Aneja, 1993). Bacterial colonies on Marine agar (Hi Media) obtained through serial dilution technique were counted by colony counter. The total population of bacteria and fungi 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 and fungi used in this study (Sokal & Rohlf, 1995).

RESULTS

The data recorded for the population count in phyllosphere of different mangrove plant

Mangrove species	No. of bacteria ($\times 10^3$)		No. of fungi ($\times 10^3$)	
	Mean	SD	Mean	SD
Acanthus ilicifolius	12.8	8.8	0.8	0.3
Aegiceras corniculatum	15.2	2.6	1.8	1.2
Avicennia officinalis	4.7	1.4	0.3	0.2
Caesalpinia crista	17.1	4.5	3.4*	0.8
Crinum defixum	528.3*	83.4	0.6	0.2
Derris heterophylla	12.6	4.3	0.3	0.2
Excoecaria agallocha	23.5	12.5	0.3	0.2
Heritiera fomes	2.3	0.2	0.4	0.2
Sesuvium portulacastrum	3.2	0.2	0.8	0.4
Sonneratia caseolaris	17.3	4.5	0.8	0.3
Tamarix troupii	11.0	1.4	3.2*	0.4

Table 1. Microbial population per cm^2 in phyllopsphere (mean and SD) of mangroves grow in intermediate salinity zone of Bhitarkanika (*, significant at $P<0.001$, $n = 3$).

species exhibited variation among different plant systems with respect to their association with microbial system. The mangrove plants of intermediate salinity zone were found to be populated with less number of bacteria and fungi as compare to low salinity zone (tabs. 1, 2). However, the leaves of *C. defixum* collected from intermediate salinity zone have shown highest bacterial population (528.3×10^3 cm^{-2}) (tab. 1). Other plant species have exhibited similar bacterial population ranged between 11-17×10^3 cm^{-2} except in *A. officinalis*, *H. fomes* and *S. portulacastrum* that had low population count of bacteria in their phyllosphere (tab. 1). A similar pattern of fungal population was observed in phyllosphere of *C. crista* (3.4×10^3 cm^{-2}) and T. troupii (3.2×10^3 cm^{-2}) (tab. 1).

The microbial population of mangrove plants of low salinity zone was found at higher level (tab. 2). Almost all plant species studied have more or less similar range except *K. candel* and *B. tersa* that exhibited higher bacterial population i.e. 167.7×10^3 cm^{-2} and 71.6×10^3 cm^{-2}, respectively (tab. 2). The fungal population in leaves of *A. officinalis*, *B. gymnorrhiza*, *B. parviflora* and *D. heterophylla* were found to be higher in the low salinity zones (tab. 2).

Among the phyllosphere of mangrove species tested, the bacterial and fungal populations in both the intermediate and low salinity zones were found significantly variable at 0.05% level. Analysis of variance for bacterial population of *C. defixum* collected from intermediate salinity zone revealed the significant variation (tab. 1). On the other hand, the rest of the plants exhibited almost similar pattern of bacterial population. Similarly, the fungal population was significantly higher in *T. troupii* and *C. crista* as compared to other plants obtained from intermediate salinity zone. A wide level of variation was found through the analysis of variance for bacterial population in phyllosphere of mangrove species of low salinity zones among them all showed significant variations ($P<0.001$) and highest number of bacterial colonies were observed in *K. candel* and *B. tersa*. Data recorded for fungal population in mangrove plants of low salinity zones were also subjected to statistical analysis. It was observed that *A. officinalis*, *B. gymnorrhiza*, *B. parviflora* and *D. heterophyla* exhibited significantly higher but similar pattern of fungal population.

Seven plant species were found common in both the salinity zone. It was observed that *A. ilicifolius*, *A. corniculatum*, *A. officinalis*, *D. heterophylla* and *H. fomes* were found to be less populated with bacteria in intermediate than low salinity where as *E. agallocha* and *S. caseolaris* had similar bacterial population in

Mangrove species	No. of bacteria (× 10^3)		No. of fungi (× 10^3)	
	Mean	SD	Mean	SD
Acanthus ilicifolus	18.7	2.5	0.9	0.4
Acrostichum aureum	32.0	15.4	0.5	0.0
Aegiceras corniculatum	28.6	12.6	1.7	0.5
Aglaia cucullata	8.1	7.6	2.0	0.5
Avicennia officinalis	18.9	1.9	3.5*	0.3
Bruguiera gymnorrhiza	27.4	3.5	3.4*	0.8
Bruguiera parviflora	22.3	3.2	3.5*	0.5
Brwonloia tersa	71.6	64.3	0.8	0.3
Dalbergia spinosa	39.7	28.0	0.4	0.2
Derris heterophylla	28.0	4.7	3.9*	0.8
Excoecaria agallocha	23.4	2.3	2.2	0.6
Heritiera fomes	18.0	2.3	0.5	0.3
Kandelia candel	167.7*	43.6	0.3	0.2
Sonneratia caseolaris	11.4	7.6	1.0	0.3

Table 2. Microbial population per cm^2 in phyllopsphere of mangroves grow in low salinity zone of Bhitarkanika (*, significant at $P<0.001$, $n = 3$).

both the salinity zones (tab.s 1, 2). Similarly, *A. officinalis*, *D. heterophylla* and *E. agallocha* have poor fungal population in intermediate salinity zone where as it was quite higher in the same plants found in low salinity area (tabs. 1, 2).

DISCUSSION

Many bacteria and fungi are reported from different mangrove ecosystem and found to be responsible for the biodegradation of organic wastes developed through mangrove litter fall. The present study on the occurrence and distribution of bacterial and fungal population in phyllosphere of different mangrove species frequency of fungal occurrence was has also supported with the observation on microbial communities of *Avicennia* sp. by Sarma *et al*. (2001). Our observations are also corroborated with the report on diversity of fungal colonies on *B. gymnorrhiza* (Maria & Sridhar, 2002).

The poor colonization of fungi on mangrove plants of intermediate salinity zone may be due to salt excretion in leaves which serve as an important defense against fungal attack and /or colonization (Gilbert *et al*., 2002). Overall, more number of bacteria and fungi were found in low salinity zone. The difference in occurrence of bacteria and fungi in different salinity zones indicates that marine microbes have a significant role to play in local mangrove communities (Steinke & Lubke, 2003). It is quite apparent from this study that no mangrove phyllosphere was populated with both bacteria and fungi with equal strength. *Crinum defixum* was populated heavily with bacteria where as fungal population recorded in this plant was very poor. Similarly, very poor population count of bacteria was observed in *T. troupii* while higher fungal population colonized it. These observatory facts indicated the biological competition among the different groups of microbial communities. Compared to studies on microbial diversity in different ecosystem, the study of microbial system in mangrove is in its preliminary phase. In this case, the information concluded by the present study may be helpful in sampling of research materials for various objectives.

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