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Relationship Between Ecological Parameters and Marine Bacteria From Mangrove Sediments of South East Coast of India.

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ABSTRACT

The marine microbes might have better potential than their terrestrial counterparts. This is due to essential nutrients, amino acids, vitamins and minerals that are available in the marine biotopes for nurturing the organisms. Hence, in the present study, we investigated the lactobacilli from the rhizosphere soil of rare mangrove species from different biotopes of east coast of India to delineate the spatial and temporal variations of lactobacilli with regard to physico-chemical variables. Ecological studies on marine lactobacilli from mangrove sediment of east coast of India. Samples were drawn from four mangrove areas of east coast of India namely Sundarbans (West Bengal), Bhittarkanika (Orissa), Coringa (Andhra Pradesh) and Pichavaram (Tamil Nadu). The sampling was made from root soil (Rhizosphere soil) of six rare mangrove species from 12 sites. Fifteen physico-chemical parameters were analyzed in the sampling sites. The count lactobacilli were isolated from the soil samples and enumerated. The lactobacilli counts in these study areas varied significantly (p>0.05) with a range from 3.0×102 to 3.1 ×104 CFU g-1. The counts were recorded maximum with Hereteira fomes at Sundarbans and minimum in Rhizophora annamalayana at Pichavaram. The monthly variation of lactobacillli counts in rhizosphere soil of R. annamalayana, the only endemic species of India, varied from 3.0×102 to 9.9×102 CFU g-1. The maximum count was observed during the post monsoon month (Nov.) and the minimum in the summer month (May)

KEYWORDS	Marine microbes, Lactobacillus, Hereteria fomes, Rhizophora annamalayana, Nutrients.

Introduction

The sea, especially in coastal areas is colonized with rich marine life due to the nutrient sources necessary for growth of the organisms especially heterotrophic microbes. The marine bacteria so far identified belonging mostly to the species of *Micrococcus, Pseudomonas, Vibrio, Flavobacterium Alcaligenes, Xanthomonas Acromobacter* and *Lactobacillus* (Gauthier *et al,* 1975; Austin, 1989; Bernana *et al,* 1997; Jayanth *et al,* 2002). The marine lactic acid bacteria have been identified as part of the normal flora of marine and fresh water fishes (Kavasnikov *et al,* 1976). Isolation and taxonomic studies of lactic acid bacteria from the marine environments to-date are only few and have generally been confined to those from cultured fish (Ringo and Gatesoupe, 1998; Gatesoupe, 1999). Franzmann *et al,* (1991) isolated two species of lactic acid bacteria of possible seawater origin from the waters of Ace Lake in Antarctica (Masuda *et al,* 1988). A novel marine lactic acid bacterium namely *Marinilactibacillus psychrotolerans* has been described for eight strains isolated from living and decomposed marine organisms collected from Japan (Morio *et al,* 2003). Lactic acid bacteria are beneficial forms that are known for their gut floral maintenance, fermentative activity, and preservative capacity, and they may have potential application values as probiotics, food preservatives, fermentation, bio-degradation, pharmaceutical and other biotechnological applications. The marine strains might have better potential than their terrestrial counterparts. This is due to essential nutrients, amino acids, vitamins and minerals that are available in the marine biotopes for nurturing the organisms (Kandler and Weiss, 1986; Havenaar *et al,* 1992a). Hence, in the present study, we investigated the lactobacilli from the rhizosphere soil of rare mangrove species from different biotopes of east coast of India to delineate the spatial and temporal variations of lactobacilli with regard to physico-chemical variables.

Materials and Methods

Sample Collection and Analysis of physicochemical parameters

Sediments samples were collected different depths from different sites of the rhizosphere soil of east coast of India. The collected samples analysis of organic matter, nutrients, major elements, trace elements and heavy metals. The physico-chemical and biological variables of the samples were analyzed using standard procedures. (Temperature, Hydrogen ion concentration (*p*H), Salinity (Jackson, 1976), Total dissolved Solids (TDS), Dissolved oxygen (Strickland and Parsons, 1972), Total nitrogen (Kjeldhal method (Jackson, 1971), Total phosphorus (Murphy and Riley, 1962), Total amino acids (Moore and Stein, 1948), Total sugars (Dubaois *et al,* 1956), Tannins (Hagerman and Butler, 1978), Total organic carbon (El.Wakeel and Riley, 1956), Estimation of Potassium in sediment, Analysis of heavy metals in sediments (Chester and Hughes, 1967).

Microbiological analysis

The plant roots and other debris were removed from the sediment samples and brought immediately to the laboratory in iced condition, maintained at 4°C and all the microbiological analysis was made within 4-6 hours of sampling. A known weight of sediment (1 g) was aseptically weighed and transferred to a stopper (150-mL) sterile conical flask containing 99-mL of sterile diluent. The sediment- diluent mixture was agitated by means of mechanical shaking for about 5-10 minutes and later it was subjected to LAB examination.

Isolation and enumeration of Lactobacilli and Total Heterotrophic Bacteria (THB)

Samples were serially diluted up to 10^{-5} with sterilized 50% seawater and plated with deMan- Rogosa- Sharpe (MRS) medium for lactobacilli and Zobell marine agar for total heterotrophic bacteria (THB). For plating, one milliliter of the serially diluted samples of sediment was pipetted out into sterile Petri-dish. Then sterile media was poured into dishes aseptically and swirled for thorough mixing. After solidification, the plates were incubated in an inverted position at 28±2°C. All the determinations were carried out in duplicates. After the incubation period, microbial colonies were counted. The counts are expressed as Colony Forming Unit (CFU) per gram of the sediment sample.

Statistical analysis

Statistical analysis was done by Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT).

RESULTS

The water salinity in the study areas varied from 6 to 25 ppt. The maximum was recorded in Pichavaram and the minimum in Sundarbans. Soil salinity in the study areas varied from 7.6 to 22.3 ppt. The maximum was recorded in pichavaram and the minimum in Sundarbans. In general, the soil salinity was comparatively higher than water salinity (Table 1). In pichavaram, monthly variations of the salinity ranged from 1.60 to 39.5 ppt in the rhizosphere soil of *R. annamalayana,* the only endemic species of India. The maximum range was observed during summer month (May) and the minimum in monsoon month (Oct.) (Table 4). Temperature in the study areas varied from 22 to 33° C in both water and sediment samples, with the maximum in pichavaram and the minimum in Sundarbans. In pichavaram, the soil temperature was higher than water temperature (Table 1). In pichavaram, monthly variations of the temperature ranged from 26.4 to 33.5° C in the rhizosphere soil of *R. annamalayana*. The maximum range was observed during summer month (May) and the minimum in monsoon month (Oct.) (Table 4). In general the *p*H did not vary between the sampling sites. Water *p*H in the study areas varied from 7.7 to 8.5. The maximum was recorded in Pichavaram and the minimum in Sundarbans. Soil *p*H varied between 7.7 and 8.3 with the maximum recorded in Bhittarkanika and the minimum in Sundarbans (Table 1). In pichavaram, monthly variations of the *p*H ranged from 7.4 to 8.9 in the rhizosphere soil of *R. annamalayana*. The maximum range was observed during summer month (May) and the minimum in monsoon month (Oct.) (Table 4). Total dissolved solids in the study areas varied from 4.430 to 6.069 ppt. The maximum was recorded in Sundarbans and the minimum in Pichavaram (Table 1). In pichavaram, monthly variations of the Total dissolved solids ranged between 2.23 and 22.8 ppt in the rhizosphere soil of *R. annamalayana*. The maximum range was observed during summer month (May) and the minimum in monsoon month (Oct.) (Table 4). Water holding capacity of sediment in the study areas varied from 6 to 8 $mL.10^{-1}g$. The maximum was recorded in Coringa and the minimum in Pichavaram (Table 1). Total amino acids of sediments in the study areas varied from 9.32 to 11.6 $mg.g^{-1}$with the maximum, recorded in Sundarbans and the minimum in Pichavaram (Table 2). In pichavaram, monthly variations of total amino acids ranged from 4.78 to 9.71 $mg.g^{-1}$ in the rhizosphere soil of *R. annamalayana*. The maximum range was observed during monsoon month (Nov.) and the minimum in summer month (May) (Table 4). Total organic carbon in the study areas varied from 1.51 to 4.53 mg C. g^{-1}. The maximum was recorded in Sundarbans and the minimum in Pichavaram (Table 2). In pichavaram, monthly variations of the total organic carbon ranged from 1.38 to 5.96 mg $C.g^{-1}$ in the rhizosphere soil of *R. annamalayana*. The maximum range was observed during monsoon month (Nov.) and the minimum in summer month (May) (Table 4). Tannins of sediments in the study areas varied from 0.41 to 1.76 $mg.g^{-1}$. The maximum was recorded in Pichavaram and the minimum in Sundarbans (Table 2). In pichavaram, monthly variations of the tannins ranged from 0.87 to 5.12 $mg.g^{-1}$ in the rhizosphere soil of *R. annamalayana*. The maximum range was observed during summer month (May) and the minimum in monsoon month (Nov.) (Table 4). Total sugars in the study areas varied from 0.43 to 0.99 $mg.g^{-1}$. The maximum was recorded in Sundarbans and the minimum in Pichavaram (Table 2). In pichavaram, monthly variations of the total sugars ranged from 0.09 to 0.58 $mg.g^{-1}$ in the rhizosphere soil of *R. annamalayana*. The maximum range was observed during monsoon month (Nov.) and the minimum in summer month (May) (Table 4). Total nitrogen of sediments in the study areas varied from 8.61 to 11.7 $g.m^{-2}$. The maximum was recorded in Sundarbans and the minimum in Pichavaram (Table 2). In pichavaram, monthly variations of the total nitrogen ranged from 3.91 to 10.2 $g.m^{-2}$ in the rhizosphere soil of *R. annamalayana*. The maximum level was observed during monsoon month (Jan.) and the minimum in summer month (June) (Table 4). Total phosphorus of sediments in the study areas varied from 3.11 to 9.3 $g.m^{-2}$. The maximum was recorded in Sundarbans and the minimum in Pichavaram (Table 2). In pichavaram, monthly variations of the total phosphorus ranged from 0.39 to 4.42 $g.m^{-2}$ in the rhizosphere soil of *R. annamalayana*. The maximum level was observed during post- monsoon month (Jan.) and the minimum in summer month (May) (Table 4). Potassium of sediments in the study areas varied from 2.35 to 5.33 $g.m^{-2}$. The maximum was recorded in Sundarbans and the minimum in Pichavaram (Table 2). In pichavaram, monthly variations of potassium ranged from 3.23 to 0.79 $g.m^{-2}$ in the rhizosphere soil of *R. annamalayana*. The maximum level was observed during post-monsoon month (Jan.) and the minimum in summer month (May) (Table 4). Analysis of heavy metals such as Copper, Iron, Manganese, and Zinc in sediment samples revealed that ferrous was the dominant one followed by manganese, copper, and zinc. The iron in the study areas varied from 14,003 to 15,143 ppm. The maximum was recorded in Pichavaram and the minimum in Sundarbans (Table 3). In pichavaram, monthly variations of the iron ranged from 4,435 to 17,450 ppm in the rhizosphere soil of *R. annamalayana*. The maximum level was observed during monsoon month (Dec.) and the minimum in summer month (May) (Table 4). Manganese in the study areas varied from 122 to 185 ppm with the maximum recorded in Pichavaram and the minimum in Sundarbans (Table 3). In pichavaram, monthly variations of the manganese ranged from 98 to 187 ppm in the rhizosphere soil of *R. annamalayana*. The maximum level was observed during monsoon month (Dec.) and the minimum in summer month (May) (Table 4). Copper in the study areas varied from 11.5 to 16.6 ppm with the maximum recorded in Pichavaram and the minimum in Sundarbans (Table 3). In pichavaram, monthly variations of copper ranged from 5.37 to 16.6 ppm in the rhizosphere soil of *R. annamalayana*. The maximum level was observed during monsoon month (Dec.) and the minimum in summer month (May) (Table 4). Zinc in the study areas varied from 38.2 to 53.5 ppm with the maximum recorded in Pichavaram and the minimum in Sundarbans (Table 3). In pichavaram, monthly variations of zinc ranged from 8.56 to 51.2 ppm in the rhizosphere soil of *R. annamalayana*. The maximum level was observed during monsoon month (Dec.) and the minimum in summer month (May) (Table 4).

MICROBIAL STUDIES

Marine lactobacilli counts in the study areas varied with a range from 3.0×10^2 to 310 $\times10^2$ CFU. g^{-1} at different sediments in depths. In general the counts were higher at 10-15 cm depths of sediments than other depth (0-5, 6-10). The lactobacilli counts were recorded maximum with *Heritiera fomes* at Sundarbans and minimum in *Rhizophora annamalayana* at Pichavaram (Table 5). In pichavaram, monthly variations of the lactobacilli counts ranged from 3.0×10^2 to 9.9×10^2 $CFU.g^{-1}$ in the rhizosphere soil of *R. annamalayana*. The maximum count was observed during monsoon month (Nov.) and the minimum in summer month (May) (Table 7). THB counts of mangrove rhizosphere soil in the different study areas varied with a range from 2.1 $\times10^3$ to 3.4×10^7 CFU g^{-1} at different sediment depths. In general, the counts were higher at 0-5 cm than other depths (6-10 and 11-15). The counts were recorded maximum with *Hereteira fomes* at Sundarbans and minimum in *Scyphiphora hydrophyllacea* at Coringa (Table 6).

Discussion

Microbial diversity comprises of a wide range of microbes than any other living organisms of the world. This rich diversity is due to existence of microbes in all niches where life is possible. They are involved in primary production, decomposition, nutrient recycling, trace gas production and other processes in coastal mangrove forest ecosystems (Kathiresan, 2000). The mangroves produce enormous amount of litter and saprophytic microorganisms to decompose the litter. The microbial processes control the transformation of nutritionally poor mangrove leaf litter to a protein-rich food substrate which represents a significant contribution to energy and nutrient budgets of the mangrove ecosystems (Heald, 1971; Odum, 1971; Fell and Master, 1973**)**. A lot of physico-chemical parameters are significantly influencing the microbial activity in the mangrove environment (Pool *et al,* 1975). the optimal growth conditions for bacteriocin production were found to be at pH 9, 35ºC, 2% salinity, glucose-1.0% as carbon source, 0.5% sodium nitrate as nitrogen source with 24hrs incubation (Rajesh Singh *et al*., 2013).

Microbial diversity in Indian mangroves have been studied especially for nitrogen fixers (azotobacter and azospirillum), phosphate solublizers, cyanobacteria, actinomycetes and fungi (Kathiresan. 2000; Kathiresan and Bingham 2001 and Kathiresan and Masilamani Selvam, 2005). However, microbial studies for lactobacilli are almost non-existent in this country. The genus *Lactobacillus* is a heterogeneous group of microorganisms which are beneficial forms of microflora of the mouth, intestinal and uro-genital tracts of mammals and can also be isolated from soil, decaying plant material of both fresh and estuarine waters (Molin *et al,* 1993). The present study proved the presence of lactobacilli in all the mangrove habitats of east coast of India. In the present study, the lactobacilli counts were higher at sub-surface than surface sediments (Table 5). This is because of the fact that the lactobacilli are anaerobic organisms, which can tolerate the aerobic condition. Anaerobic bacteria like lactobacilli which are present sub-surface of region of sediment carry out most decomposition, except where animal burrows and plant roots change oxygen to deeper sediment layer. Anaerobic decomposition in mangrove sediment is performed by a wide variety of bacterial types including lactobacilli that utilize a number of electron acceptors to oxidize carbon (*e.g.,* nitrate, oxidized iron, manganese and sulfate) (Anderson and Domesh, 1975).

t is interesting to note from the present study that different mangrove species harboured varied quantities of lactobacilli although growing in the same location. *Hereteira fomes* supported the maximum counts of lactobacilli followed by *Xylocarpus mekangenesis, Nypa fruticans, Scyphiphora hydrophyllacea,* and *Rhizophora annamalayana* exhibited the minimum counts (Table 5). This differential microbial counts may be attributed a rhizosphere effect which varies with mangrove species. The effect of root exudations which include both promoters (Sugars, amino acids, etc.) and inhibitors (phenolic compounds) and the ratio between the two types of species of compound influence the microbial growth and multiplication (Ravikumar and Kathiresan, 1993).In the present study, the lactobacilli species significantly varied with places. In general, all the mangrove species could support the lactobacilli with their population high in sundarbans and low in pichavaram. This variation can be attributed to the ecological conditions of the mangrove habitats, especially the nutrient levels. The mangrove creeks are considered as the major conducts for tidal exchange of dissolved and particulate matter between the forest and adjacent coastal waters (Wolanski *et al,* 1992). Exports of dissolved inorganic nutrients by tidal waters in the mangrove forest where the forest floor and creek bottom are known to be sink of nutrients (Alongi 1994). Human activities also influence the microbial load in different places. Sundarbans receives high loads of nutrients through river run-off and also higher load of wastewater discharges, which enhance the growth of microbes (Table 5). In pichavaram, monthly variation of the lactobacilli population varied from 3.0×10^2 to 9.9×10^2CFU. g^{-1} in the rhizosphere soil of *R. annamalayana.* The maximum count was observed during monsoon (Nov.) and the minimum in summer (May). This may be due to nutrient inflow from terrestrial region through flooding due to heavy rain during mansoon (Kathiresan, 2000). Ecological parameters are mainly constituted by bioclimatic criteria and edaphic variables. This general assumption is valid for terrestrial as well as for estuarine ecosystems. This means that whenever we establish ecological relationships, we try for instances to quantify the values of environmental parameters which are responsible for the presence of the biological systems. Mangroves seem to remain almost constant all over the world in terms of phenological, morphological, anatomical and physiological adaptive characters, even under very distinct ecological localities (Kathiresan and Binghm, 2001). The present study found that physicochemical parameters varied significantly between the study areas of east coast of India and positively correlated with important nutrients. Hence lactobacilli counts varied, based on the nutrients and ecological impacts of different biotopes of the present study areas.

Lactobacilli relation to heavy metals

Recent industrialization of various tropical regions resulted in significant accumulation of pollutants into mangrove environment (Silva *et al,*1990 and Lacerda *et al*., 1993). The heavy metals have received special attention, due to their long term effects on the environment and their properties of accumulation in protected depositional environments where mangroves are also best developed (Harbison, 1981; Silva *et al,* 1990). The trace metal pollution has resulted in increased concentration in many natural environments, subjecting the biota to toxic levels to the pollutants and leading to reduction in microbial biodiversity. The trace metals in the present study significantly varied with study areas (Table 3) and they were negatively correlated to lactobacillus population. In pichavaram, the lactobacilli counts were found lower than other study areas and this conjugated with high levels of trace metals in pichavaram sediments.

Summary and Conclusion

The mangroves are growing at extreme conditions of marine environment, the microorganisms present in that environment should be highly tolerant of the extreme conditions. Lactobacilli counts increase significantly with decreasing salinity, increased levels of nitrogen, phosphorous, potassium as well with increased levels of THB, and copper. However, the lactobacilli counts decreases significantly with increasing levels of manganese, zink and iron. In conclusion, this study clearly demonstrates that the lactobacilli and THB have been present in the different environmental conditions, Therefore, we expected that the microorganisms in the mangrove soil could be the potential source of bioactive secondary metabolites, that will be used for new drugs in the emerging field of pharmacy filed.

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