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RESEARCH ARTICLE

BIODIVERSITY OF HALOPHILIC PHOSPHOBACTERIA IN MANAKKUDI MANGROVE IN RELATION TO ENVIRONMENTAL FACTORS

*[1]Ravikumar, S., [1]Shanthy, S., [1]Kalaiarasi, A., [1]Palaniselvan, G. and [2]Sumaya, M.

[1]School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi – 623 409, Ramnathapuram District, Tamil Nadu, India.
[2]Thassim Beevi abdul kader college for women, Kilakarai- 623 517, Ramanathapuram District, Tamil Nadu, India.

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ABSTRACT

Biodiversity of halophilic phosphate solubilizing bacteria were assessed from root, rhizosphere soil, non-rhizosphere soil and water from Manakkudi mangrove ecosystem of South West Coast of India. Nine phosphobacterial species *Bacillus subtilis, Bacillus megaterium, Micrococcus roseus, Bacillus cereus, Escherichia coli, Arthrobacter illicis, Pseudomonas aeruginosa, Enterobacter aerogenes* and *Micrococcus luteus* were identified. The root samples exhibited higher counts of Phosphate solubilizing bacteria as compared to the rhizosphere soil samples which is influenced by the pH and salinity.

INTRODUCTION

Mangrove ecosystem in the marine environment is unique in the embracing a plthera of microbial population especially Phosphate solubilizing bacteria which are very important due to their in the degradation of organic matter and nutrient regeneration (Chapman, 1984). A number of static and dynamic biological, physico-chemical factors are known to influence the development and stability of mangrove community. These factors and their interactions play a significant role in the nutrient flows in the system and it is necessary to understand various processes interacting with them (Kathiresan and Bingham, 2001). The addition to mangrove plants some associated plant species are also found beyond the mangrove environment. These associated plants have interactions in terms of enhancing nutrient availability and protecting the mangrove plants from natural disaster. Many species of phosphate solubilizing bacteria associated with mangrove roots, rhizosphere sediments were reported by Vazquez *et al.* (2000) and Kathiresan and Selvam (2006). The mechanism responsible for microbial phosphate solubilization in mangrove ecosystem is considered to involve the production of several organic acids (Vazquez *et al.,* 2000). A few studies have demonstrated the ability of phosphate solubilizing bacteria associated with roots of *Avicennia germinans* L., *Languncularia racemosa* L., (Vazquez *et al.,* 2000) ans rhizosphere soil (Kathiresan and Selvam 2006; Kothamasi *et al.,* 2006) to solubilize insoluble forms of phosphates. But such studies are lacking in the Manakudy mangrove ecosystem of Palk Strait region. Hence, the present study was done to biodiversity of halophilic phosphobacteria in Manakkudi mangrove ecosystem.

***Corresponding author:** ravibiotech201321@gmail.com

MATERIALS AND METHODS

Sample collection

Root, rhizosphere soil, non-rhizosphere soil and water samples were collected monthly from 2 mangrove species *viz., Avicennia officinalis* L., *Rhizosphora mucronata* Poir. and 3 associated mangrove species *viz., Panicum repens* L., *Hymenacene acutigluma* S. and *Acrostichum aurecum* L. from Manakkudi mangrove ecosystem (Lat. 8° 21′ N and Long. 77° 30’E). The samples were transferred to the laboratory immediately and total counts of PSB from collected samples were enumerated. Isolation and identification of phosphate solubilizing bacteria. All the samples were subjected for Pikovkya’s medium (glucose: 10g; tricalcium phosphate: 5g; NH_4SO: 0.5g; $MgSO_4.7H_2O$: 0.1g; KCl: 0.2g; $MnSO_4$: trace; $FeSO_4$: trace; yeast extract: 0.5g; Agar: 15.0g; aged seawater: 500ml; distilled water: 500ml; pH 7.2±0.2; autoclaved at 15lbs for 15 min). The plates were incubated at 28±2°C for 7 days. Morphologically different phosphobacterial species were identified by repeated streaking and identified by Bergey’s Manual (Holt *et al.,* 1994).

Environmental factors

Various physico-chemical parameters viz., pH, EC, total nitrogen (Subbiah and Asija, 1956), phosphate (Olsen et al.

1954), potassium (Guzman and Jimenez, 1952), organic carbon (El Wakeel and Riley, 1956), iron, copper, zinc and manganese (Lindsay and Norwell, 1978) were analysed in the rhizosphere soil, non-rhizosphere soil and water samples. The water samples were subjected for the analyses of nitrite, nitrate, bicarbonate, chloride, sulphate, calcium and magnesium following the methods of American Public Health Association (1985).

RESULTS

The Phosphate solubilizing bacteria counts varied from 0.650 to 475.86 CFUx10^4.g^{-1} in roots (Fig. 1), 2.22 to 66.34 CFU x 10^4.g^{-1} in rhizosphere soil samples (Fig. 2). 1.05 to 14.55 CFU x 10^4.g^{-1} in non-rhizosphere soil, 0.42 to 12.96 CFU x 10^4.g^{-1} in water sample (Fig. 3). Among the plant species, the maximum counts were recorded in *Hymenachene acutigluma* in July and minimum in *Avicennia officinalis* in December. The level of pH varied (from 2.9 (June) to 7.2 (January) in rhizosphere soil, 4.6 (May) to 7.8 (July) in non-rhizosphere soil and 6.50 (July) and 8.30 (January) in water sample (Fig4 and 5). The EC in the rhizosphere soils ranged from 1.1 ds.m^{-1} (November) to 10.0 ds.m^{-1} (July), 0.9 ds.m^{-1} (December) to 10.0 ds.m^{-1} (September) in non-rhozosphere soil (Fig.6) and 2.5 10.0 ds.m^{-1} (July) to 21.0 10.0 ds.m^{-1} (Septemper) in water sample (Fig.7).

Table 1. Seasonal distribution of PSB counts in mangroves

Sample	*No. of PSB counts CFUx10^4.g^{-1}*		
	North east monsoon	Non monsoon	South west monsoon
Root	10.21	62.66	164.65
Rhizosphere soil	12.16	11.40	20.94
Non-rhizosphere soil	2.92	3.19	6.76
Water	0.51	3.97	6.04

(Fig.8). In water, the level of nitrite and nitrate ranged from 9.34 to 32.12 mil.eq.l^{-1} and 0.09 to 0.89 µg.g^{-1} respectively (Fig.9). The level of phosphate in the rhizosphere soil ranged from 0.20 to 4.75µg.g^{-1}. However *Hymenachene acutigluma* (July) (Fig.10) holds maximum level. The level potassium ranged from 10.70 to 40.09µg.g^{-1} however the maximum content was recorded in *Hymenachene acutigluma* (July) (Fig. 11). The total organic carbon ranged from 0.64 to 2.7% and the maximum content was found in *Hymenachene acutigluma* (June) (Fig.12). In non-rhizosphere soil, the level of phosphate varied from 0.64 to 4.25 µg.g^{-1}, potassium ranged from 13.50 to 37.00µg.g^{-1} (Fig.10), the total organic carbon ranged from 0.43 to 3.1% (Fig.12). In water, the level of phosphate ranged from 0.47 to 2.95 mil.eq.l^{-1}, potassium ranged from 1.56 to 6.72 mil.eq.l^{-1}, organic carbon from 0.13 to 1.33% (Fig. 9 & 13). The content of iron in the rhizosphere soil varied from 8.23 to 19.60 ppm however, the maximum content was found in *Rhizophora mucronata* (July) (Fig.14), copper ranged from 0.22 to 2.84 ppm and the maximum content was recorded in *Rhizophora mucronata* (October) (Fig.15), zinc ranged from 0.09 to 4.08 ppm and the maximum content was found in *Avicennia officinalis* (April) (Fig. 16), manganese ranged from 3.43 to 17.56 ppm and the maximum content was found in *Avicennia officinalis* (October) (Fig.17). In non-rhizosphere soil, iron ranged from 10.12 to 17.86 ppm (Fig.14), copper ranged from 0.40 to 1.80 ppm (Fig.15), zinc ranged from 1.05 to 3.89 ppm (Fig.16) and manganese ranged from 5.41 to 17.93 ppm (Fig.17). The bicarbonate content in water varied from 2.00 to 14.0 mil.eq.l^{-1}, chloride from 20.00 to 84.50 mil.eq.l^{-1}, sulphate from 0.12 to 18.60 mil.eq.l^{-1}, calcium from 4.00 to 12.00 mil.eq.l^{-1} and magnesium from 10.00 to 39.20 mil.eq.l^{-1} (Fig.18). Among the season, the values are found maximum during the northeast monsoon and decreasing trend was noticed from non-monsoon season to southeast monsoon season (Tables. 1&2).

Table 2. Seasonal variation of environmental factors in rhizosphere soil, non-rhizosphere soil and water samples

Parameters	*Rhizosphere soil*			*Non-rhizosphere*			*Water*		
	Northeast monsoon	Non-monsoon	South-west monsoon	Northeast monsoon	Non-monsoon	South-west monsoon	Northeast monsoon	Non-monsoon	South-west monsoon
pH	5.46	6.22	4.41	6.67	5.91	7.60	7.93	7.51	7.00
Ec (ds.m^{-1})	2.41	5.06	6.26	1.23	3.07	2.77	6.73	8.56	7.43
N (µg.g^{-1})	40.01	30.99	41.89	32.90	30.20	22.00	-	-	-
P_2O_5 (µg.g^{-1})	1.44	1.71	2.48	2.06	2.52	3.27	1.09	1.46	1.60
K_2O (µg.g^{-1})	27.82	22.17	22.87	21.33	15.54	16.93	-	-	-
HCO_3 (mil.eq.l^{-1})	-	-	-	-	-	-	2.83	5.90	3.40
Cl_2 (mil.eq.l^{-1})	-	-	-	-	-	-	72.23	36.92	21.83
SO_4 (mil.eq.l^{-1})	-	-	-	-	-	-	10.87	2.40	0.40
Ca (mil.eq.l^{-1})	-	-	-	-	-	-	10.00	6.47	5.33
Mg (mil.eq.l^{-1})	-	-	-	-	-	-	23.63	23.93	22.27
K (mil.eq.l^{-1})	-	-	-	-	-	-	4.90	2.92	2.96
NO_2 (mil.eq.l^{-1})	-	-	-	-	-	-	16.44	13.29	16.89
NO_3 (mil.eq.l^{-1})	-	-	-	-	-	-	0.49	0.25	0.67
Organic carbon (%)	1.14	1.59	2.19	1.17	1.37	0.97	0.85	0.55	0.90
Fe (ppm)	12.26	12.42	15.93	9.77	12.69	13.65	-	-	-
Cu (ppm)	1.58	1.01	1.00	1.14	1.02	0.91	-	-	-
Zn (ppm)	1.87	2.54	3.04	1.69	2.16	2.59	-	-	-
Mn (ppm)	10.77	11.16	9.96	9.20	12.13	10.95	-	-	-

Values are significant at 1% level among seasons, (-) : Not detected

The nitrogen content of the rhizosphere soil varied between 14.90 to 60.0 µg.g^{-1}. However maximum content was recorded in *Avicennia officinalis* (July) and in non-rhizosphere soil, level of nitrite and nitrate ranged from 15.40 to 51.80 µg.g^{-1}

Seventy two Phosphobacterial strains were isolated from Manakudy mangrove ecosystem, among them 9 strains were identified. *Bacillus subtilis* (18.05%) was found to be the dominant species, followed by *Bacillus megaterium* (16.66%),

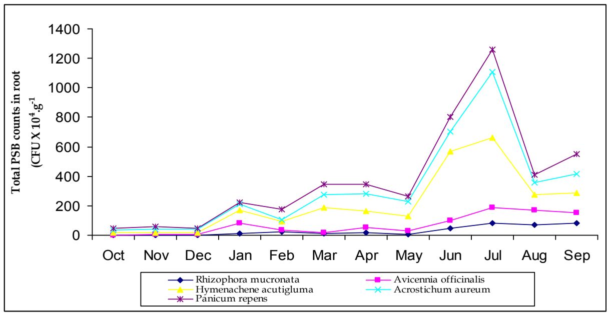


Fig. 1. Monthly variation of PSB counts in root samples

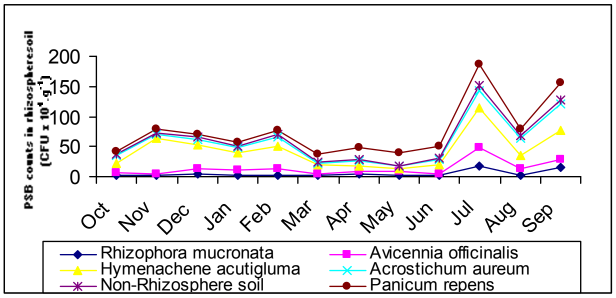


Fig. 2. Monthly variation of PSB counts in rhizosphere soil samples

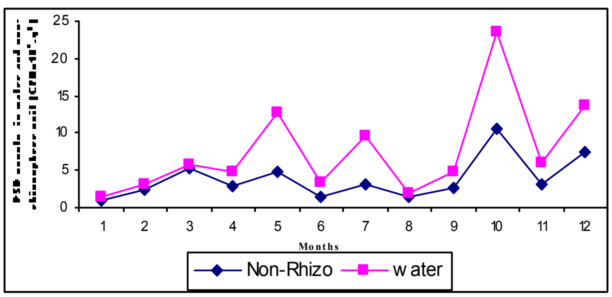
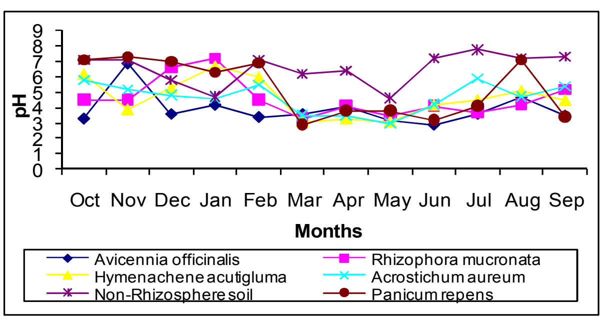


Fig. 3. Monthly variation of PSB counts in non rhizosphere soil and water samples



4. Monthly variation of pH in soil samples

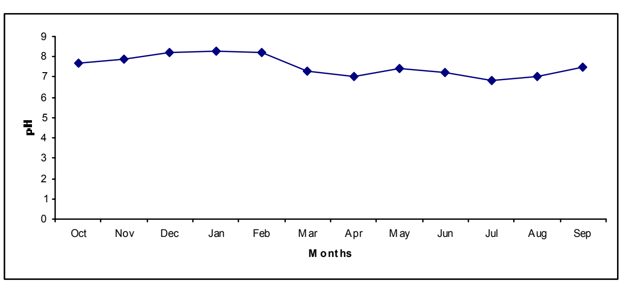


Fig.5. Monthly variation of pH in water samples

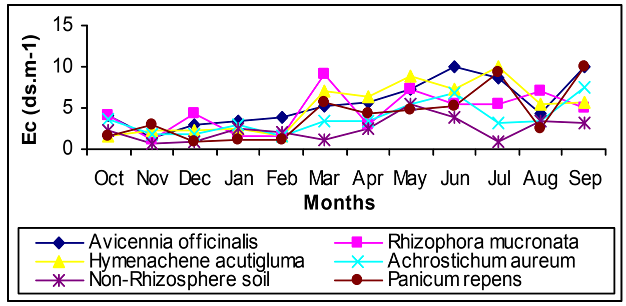


Fig. 6. Monthly variation of Ec in soil samples

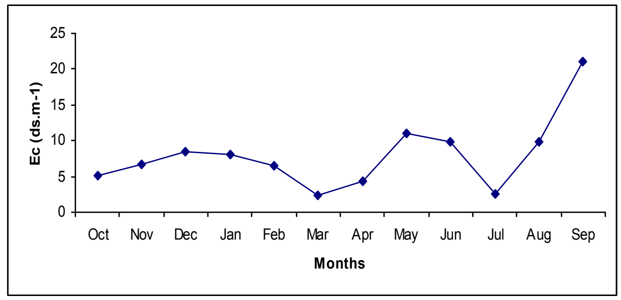


Fig. 7. Monthly variation of Ec in water samples

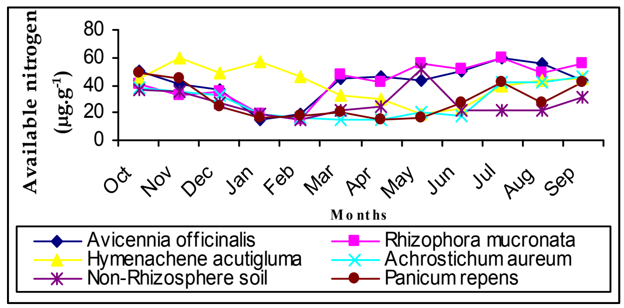


Fig. 8. Monthly variation of availabl e nitrogen in soil samples

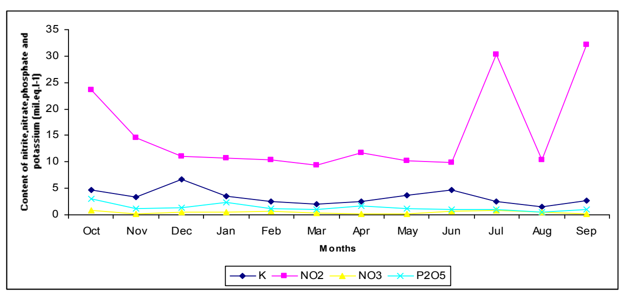


Fig. 9. Monthly variation of nitrite, nitrate, phosphate and potassium in water samples

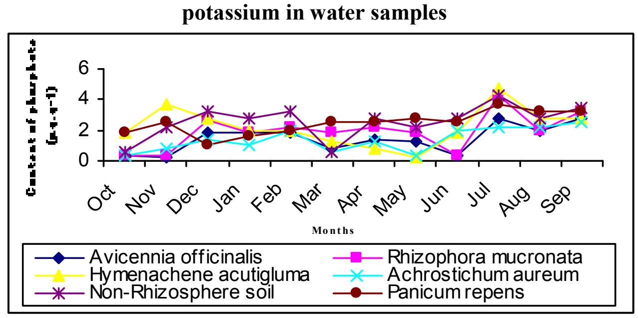


Fig.10. Monthly variation of phosphate in soil samples

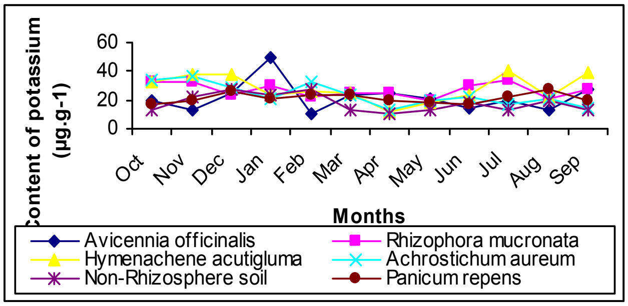


Fig. 11. Monthly variation of potassium soil samples

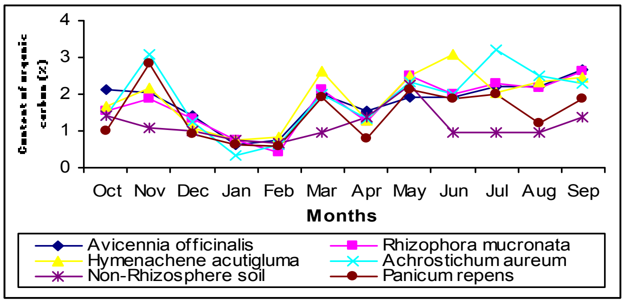


Fig. 12. Monthly variation of organic carbon in soil samples

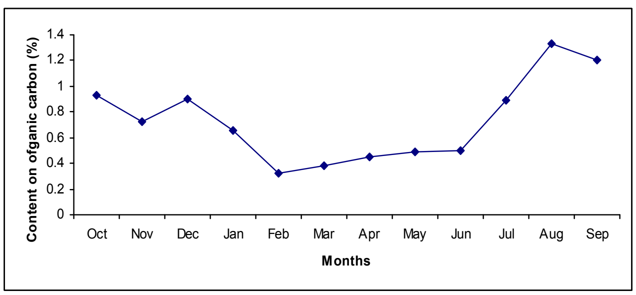


Fig. 13. Monthly variation of organic carbon in water samples

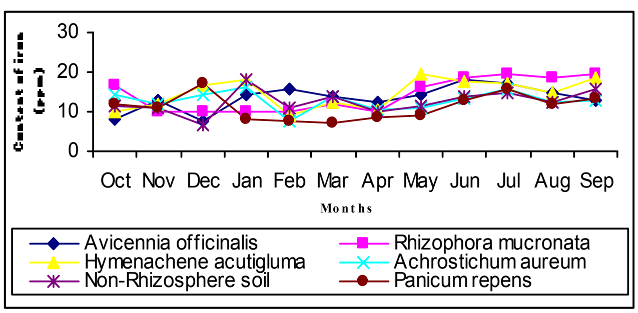


Fig.14. Monthly variation of iron in soil samples

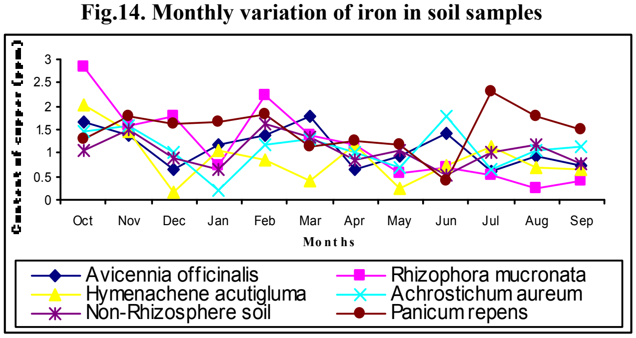


Fig. 15. Monthly variation of copper in soil samples

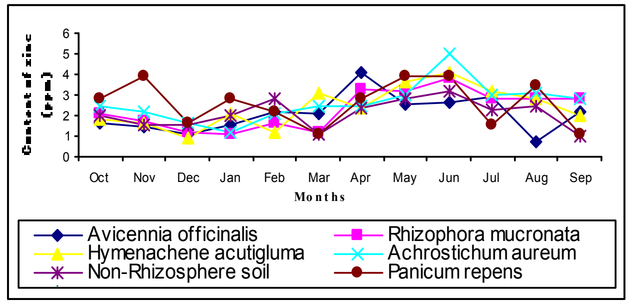


Fig. 16. Monthly variation of zinc in soil samples

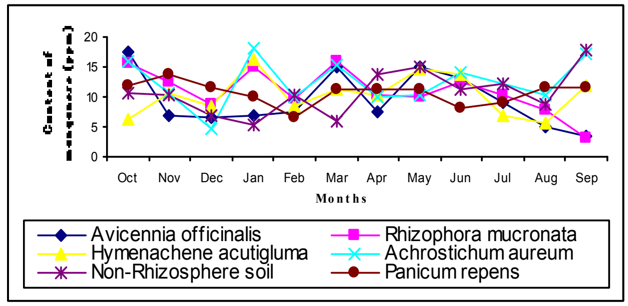


Fig. 17. Monthly variation of manganse in soil samples

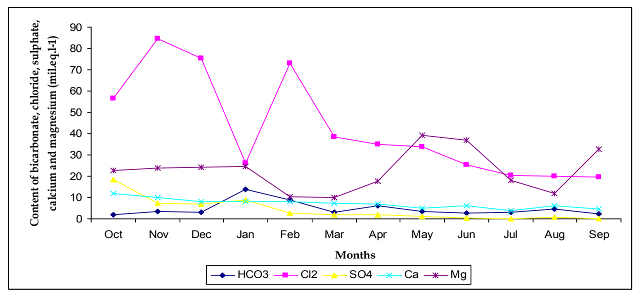


Fig.18. Monthly variation content of bicarbonate, chloride, sulphate, calcium and magnesium in water samples

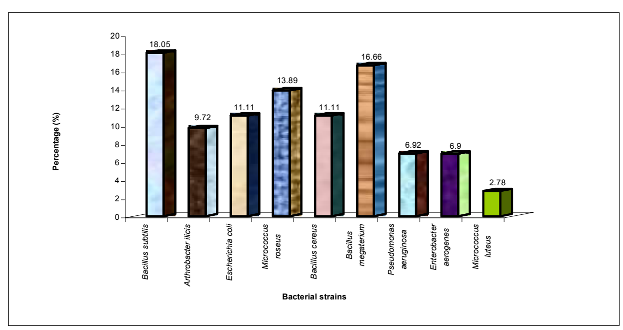


Fig. 19. Percentage composition of phosphobacteria in the Manakudy mangroves

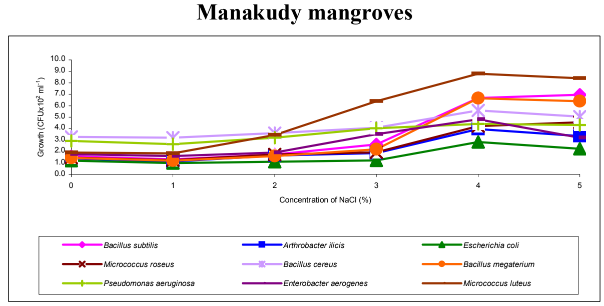


Fig. 20. Growth of PSB at different salinity

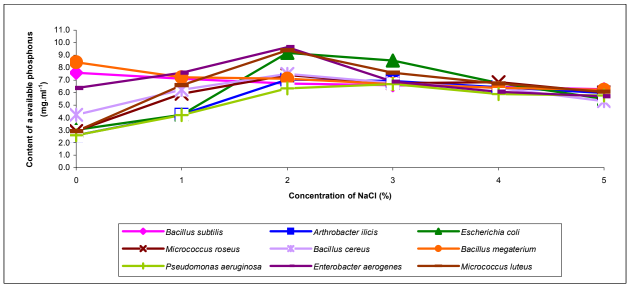


Fig. 21. Effect of NaCl on the phosphate solubilizing activity in PSB

Micrococcus roseus (13.89%), *Bacillus cereus* (11.11%), *Eschericha coli* (11.11%), *Arthrobacter illicis* (9.72%), *Pseudomonas aeruginosa* (6.92%), *Enterobacter aerogenes* (6.9%) and *Micrococcus luteus* (2.78%) (Fig.19). The growth of *Bacillus subtilis*, *Micrococcus roseus* extended their growth upto 5% NaCl. The phosphate solubilizing activity was found to be higher in 1% NaCl in *Bacillius megaterium* and *Bacilius subtilis* but other strain showed maximum activity at 2% NaCl except *Pseudimonas aeruginosa* which showed higher activity at 3%NaCl concentration (Fig. 20 & 21).

DISCUSSION

Bacteria are the predominant microorganisms that solubilize mineral phosphate in soils, as compared to fungi or actinomyces (Kucey, 1983; Yin, 1988). The root samples exhibit high rates of Phosphate solubilizing bacteria than rhizosphere soil. Biological decomposition of inorganic phosphorous in the marine environment is considered to be the result of bacterial action and the extent and rate of inorganic phosphorus by bacteria depend on the density of the phosphate solubilizing bacterial population (Vander Linden, 1988; Ayakkannu and Chandramohan, 1971). Purushothaman (1994) recorded higher PSB population density in seawater than in Veli Lake, West Coast India. The present study observed that, the phosphate solubilizing bacteria counts were found maximum in the rhizosphere of mangrove associated plants than in mangrove plants. This might be due to the extensive fibrous root system of grass species and also low tannin concentration. The minimum phosphobacterial counts in the rhizosphere of mangrove plants might be due to the higher content of tannin released from the mangroves through root exudates (Kathiresan, 1995).

Total counts of nitrogen-fixing Azospirillum enumerated from the samples of rhizosphere soil and root have been shown to have 10-fold higher in root than the soil samples (Ravikumar *et al.,* 2002). This may be attributed to the quantity and quality of root-derived carbon, which supports the biomass expansion. Vander Linden (1988), Ayyakannnu and Chandramohan (1971) and Venkateswaran (1981) reported that, occurrence and higher counts in phosphate solubilizing bacteria in marine sediments might be attributed to the rich organic carbon. Laksmana Perumalsamy (1978) reported that, the monsoonal rains enrich the coastal environment with the essential fraction of the nutrients addition of these nutrients and other organic in the sediments might have enhanced the bacterial density (Chongprasith, 1992). It is also evident from the present study that, the counts of PSB in the rhizosphere environment were found higher in Manakudy mangroves. When compared with the other mangrove ecosystem in South East Coast of India (Ravikumar *et al.,* 2004). The Phosphate solubilizing bacteria have been reported to typically produce to a variety of organic acids, which in turn solubilize inorganic phosphate (McLaughlin *et al.,* 1988; Chen *et al.,* 2006) and possibly also help in the acquisition of nutrients during the monsoonal months. In the present study, higher phosphate solubilizing bacteria population was observed and has been the result of higher nutrient load in the study area brought by land run-off. Kannapiran (1997) suggested that, the phosphate solubilizing bacteria populations occurred during the post monsoon months Gulf of Mannar region in the South East Coast of India might be due to the large amount of dissolved and particulate nutrients introduced into the monsoonal months by land run-off and rainfall and their subsequent deposition of these nutrients over the reef sediments during the next post monsoon.

Ravikumar *et al.* (2002, 2004) reported that the growth of *Azospirillum* and *Azotobacter* were found maximum at a salinity gradient of $30g.l^{-1}$, but the rate of nitrogen fixation activities are completely arrested more than $15g.l^{-1}$ salinity levels. In the present study, the maximum level of salinity in the rhizosphere soil and root favored high phosphate solubilizing bacteria counts in *Panicum repens* rhizosphere soil and *Acrostichum aurem* root. It is also observed that, the salinity was recorded during summer months and lowers during the monsoonal months. This lower salinity could be attributed by the preparation that occurred during monsoon seasons in the study area. Kannapiran (1997) reported that, higher salinity was observed during summer months and low salinity in monsoonal months in the Gulf of Mannar region of South Eastern Coast of India. Vazquez *et al.* (2000) reported thirteen species of phosphate solubilizing bacterial in two mangrove species. Comparatively, the present study identified 9 PSB strains which are less in diversity than in other mangrove species. The present study also made an attempt to find out the growth and phosphate solubilising activity of 9 phosphate solubilizing bacteria strains at varied salinity levels. It reveals that, all the species could able to growth at higher salinity levels with maximum solubilizing activity and hence it confirmed to be the pure halophilic forms. It is inferred from the present study that, the isolated phosphate solubilizing bacteria strains mass cultivated with ambient pH (7) and salt concentration (>1% NaCl) could be used for raising vigorous mangrove seedlings in the nursery during the monsoon season, so as to enable to utilize more phosphate activity of phosphobacterial isolates. It is also recommended that, further attempt to use these halophilic phosphobacterial strains for enhancing the growth and yield of rice crops are highly warranted to tackle the upcoming global warming and sea level rise

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