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Occurrence of Phosphate-Solubilizing Bacteria in Rhizospheric and Pneumatophoric Sediment of *Avicennia marina*

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Abstract
The present study deals with the isolation and characterization of phosphorus-solubilizing bacteria (PSB) from *Avicennia marina* rhizospheric sediment, pneumatophoric sediment and non-rhizospheric sediment. The sampling was carried out in four mangrove forests of Mumbai, India. The physicochemical parameters like pH, electrical conductivity, organic carbon, texture, total Phosphorus (P), inorganic P, organic P, Olsen P, iron, calcium, magnesium and zinc in sediment were analysed along with the isolation and molecular characterization of PSB. Significantly, lower pH was observed in the rhizospheric sediment than in the pneumatophoric and bulk sediment. Abundance of PSB was also found in the rhizospheric zone. This was supported by other physicochemical parameters such as high organic P, Olsen P, and higher bacterial count along with low inorganic P. In this study, 8 PSB were isolated and 16S rDNA sequence revealed that all the isolates belong to the genus *Bacillus*. Among the PSB isolates, *B. subtilis* sub. *spizizenii* TU-B-10 showed significantly higher solubilization activity (85.8±0.0 µg P released per 10^8 cfu in 72 hrs). The isolates that showed higher phosphate-solubilizing potentials can be explored as phosphatic bio-fertilizer to enhance the agricultural, aquacultural and mangrove productivity.

Keywords: Phosphorus, Phosphate-solubilizing bacteria, acid phosphatase activity, *Avicennia marina*

1. Introduction
Phosphorus (P), a major growth-limiting nutrient second to nitrogen, is one of the major essential macronutrients for plants and it is applied to soil in the form of phosphatic fertilizers in order to overcome the phosphorus deficiency. A large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and only a small proportion in the form of two soluble compounds, the monobasic ($H_2PO_4^-$) and the diabasic (HPO_4^{2-}) ions is made available to plants [19]. Phosphorus which is present in the soil fractions can be released by the slow process of mobilization. It was found that many soil microbes, especially bacteria and fungi, can work as good mobilizers of phosphorus. They help in solubilizing P from organic and inorganic matters [7]. The use of PSB can provide a solution to the problem of limited P availability in salt-affected soils, aquaculture in salt-affected inland and coastal areas. Mangroves are one of the taxonomically-diverse groups of woody shrubs, which possess ability to survive along sheltered tropical coastline in saline environments under tidal influence [18]. Mangroves are highly dynamic and complex systems that are still poorly understood. Hence the study combined with conservation actions will be necessary to preserve these fragile and unique environments [16]. The rhizosphere (true roots) and pneumatophore (respiratory root) zones of mangroves may harbour unique bacterial community than that of bulk sediments and hence with these backgrounds the study was carried out to characterize PSB in *Avicennia marina* sediments and isolated strains were tested for revealing their solubilization activity.

2. Materials And Methods
The sampling was carried out in Alibag, Mahul, Versova (Mud Island) and Gorai, Mumbai coast during 2013. The mangrove species selected for the study is *Avicennia marina*

Table 1: Details of sampling site

Sl no	Site	Coordinates	Soil Temperature (˚C)
1	Versova	19˚ 08' 25.1" N, 072˚ 47' 63.6"E	30.0
2	Mahul	19˚ 00' 80.4"N, 072˚ 53'15.1"E	28.7
3	Alibag	18˚ 38' 32.9" N,072˚ 54' 07.3"E	22.0
4	Gorai	19˚14' 23.1" N, 072˚ 49' 3.2"E	31.9

2.1. Sample Collection
Three sediment types namely bulk sediment (non-rhizosphere), rhizospheric sediment and sediment on the pneumatophores (referred as pneumatophoric sediment) were collected aseptically in sterile Uricol bottles (Hi-Media, India). Bulk sediment was collected in the depth of 0-15cm [9]. The rhizosphere sediments were collected by carefully removing the soil adhering in a 2-3 mm thickness around the individual roots of *A. marina*. The sediment at the base of the pneumatophore was collected. All the samples were kept in ice-box and transported to the laboratory immediately for further analysis. The sediment samples were subjected to physicochemical characterization and for this air dried samples were used. The results were expressed on moisture-free basis. The sediment pH and EC was measured using portable pH and EC meters (Eutech Instruments, Malaysia) in sediment and water ratio of 1: 2.5 and the sediment textures was estimated by the international pipette method [9]. The organic carbon (C) content of the sediment was estimated by Walkley and Black (1934) [21] method. Organic and inorganic P in sediment was estimated by ignition method [8].The available P in sediment was estimated using Olsen's method [12] as the sediment pH was in neutral to alkaline range. For the estimation of iron, calcium, magnesium and zinc, the sediment samples were digested using microwave based digestion system (Multiwave 3000, Anton Parr, USA) and the digested samples was analysed by atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer,USA) using flame atomization.

2.2. Distribution of phosphate-solubilizing bacterial count
PSB were enumerated on Pikovskaya's agar (PVK) and National Botanical Research Institute's Phosphate Growth medium (NBRIP). To avoid growth of fungal solubilizers, actidione (cyclohexidine, Hi-Media Mumbai) was added to media at the rate of 50 µg ml^{-1}. NBRIP medium was prepared for one litre in the following manner in laboratory ; glucose 10 gm, tricalcium phosphate 5 gm, $MgCl_2.6H_2O$ 5 gm, $MgSO_4.7H_2O$ 0.25 gm, KCl 0.20 gm, $(NH4)_2SO_4$ 0.10 gm, Agar 15 gm. Standard plate method and dilution procedure was followed for the enumeration.

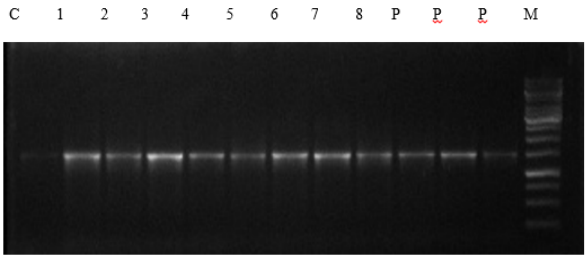
2.3. Isolation of bacterial strains
The distinct bacterial colonies were selected according to their morphological appearance and halo formation in both PVK agar and NBRIP growth media. After continuous sub-culturing, Gram staining technique was done for checking purity and the isolates were subjected to DNA extraction. The DNA extracted was analysed by agarose gel electrophoresis using 1.0% agarose gel pre-stained with ethidium bromide. The 16S rDNA of the bacterial isolates were amplified using primers 27F and 1492R [3] and were sequenced at GeNei Pvt. Ltd., Bangalore, India. The 16S rDNA sequence was searched on GenBank Database of National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) using the BLAST algorithm and the closest similarity was determined.

2.4. Phosphate-solubilizing Activity of PSB
The identified strains were tested for their ability to solubilize insoluble inorganic phosphate (Calcium hydroxyapatite). The bacterial strains grown on 1.5% nutrient agar plates were inoculated into nutrient broth and incubated for 72 hours at 28±2 °C. One ml of the broth culture was transferred to 3 ml sterilized basal medium with ingredients (per litre): Yeast extract-2 g, Dextrose-10 g, Peptone-2.5 g, and pH 8. 0.5 ml of 1% filtered and sterilized suspension of calcium hydroxyapatite was added under constant stirring. Tubes with suspension of calcium hydroxyapatite without bacterial culture served as control. The tubes were incubated for 72 hours at 28±1 ^{0}C. After that both the experimental and control tubes were centrifuged at 3000 rpm for 15 minutes. The reactive phosphate was determined spectrophotometrically using ascorbic acid method at 660 nm. The difference in phosphate concentration of the experimental and control tubes was taken as the amount of phosphate released by the cultures from the hydroxyapatite. The phosphate solubilization activity is expressed as µg P released per 10^8 cfu in 72 hrs [5].

2.5. Statistical Analysis
All statistical analysis was carried out using SPSS 16.0 (SPSS Inc.,
Chicago, Illinois, USA). The two-way ANOVA was carried out for the factors, sediment type and site. Duncan homogenous grouping of means was carried out at p =0.05 for the significant effects. One-way ANOVA was carried out for the finding the significant difference among the phosphate-solubilizing activity of the bacterial isolates.

3. Results
3.1. Analysis of physicochemical parameters
The sediment physicochemical parameters of the study sites are presented in Table 2. pH of sediment in all the four sites was found to be neutral to slightly alkaline. Sediment texture was found to be clayey in all sites except Alibag, which had sandy texture. Organic Carbon ranged from 0.90% in Alibag rhizospheric sediment to 4.29% in Mahul bulk sediment. Total P ranged from 458.08 to 1980 mg kg^{-1}. Organic P ranged 53.47 to 799.70 mg kg^{-1}. It was noted that pneumatophoric sediment had significantly lower organic P compared to bulk and rhizospheric sediment. In the present study, inorganic P ranged 381.16 to 1580.8 mg kg^{-1}. The rhizospheric sediments showed significantly lower inorganic P than bulk and pneumatophoric sediments. Olsen P in the sediments ranged from 3.0 to 13.99 mg kg^{-1} and was significantly higher in rhizospheric sediments when compared to bulk and pneumatophoric sediment. Metal content in all sediment types were noted to be higher.

3.2. Isolation of phosphate-solubilizing bacteria
21 bacterial isolates were isolated on the basis of clear zones/ Halo around colonies. Out of 21 isolates, eight could be purified using repeated streaking and Gram staining. The genomic DNA was extracted and 16S rDNA was sequenced. All the eight isolates belong to the Genus *Bacillus*. Notably, *B. pumilus*, *B. tequilensis*, and *B. mojavensis* were observed apart from *B. subtilis* and *B. atrophaeus* (Table 4).



PSB Isolates: 1-8; Marker (M): 1 Kb; Negative Control: C; Positive Control: P

Fig 1: PCR Amplification of 16S rDNA of 8 isolates of phosphate-solubilizing bacteria

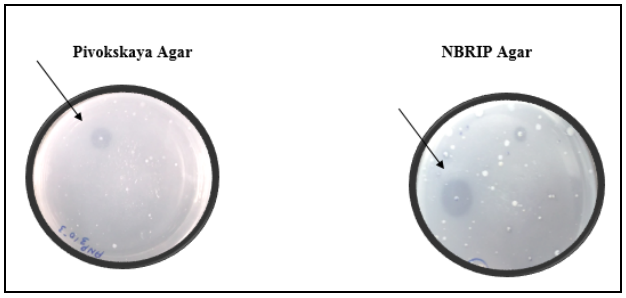


Fig 2: Phosphate-solubilizing bacteria on Pivokskaya and NBRIP agar plates (Halo-zone around the colonies indicates the solubilization of inorganic Phosphate)

Table 2: Physicochemical parameters (in range) of the sediment in the study sites

Parameters	Sediment	Alibag	Mahul	Versova	Gorai
pH	R	7.40-8.20	7.20-7.70	7.00-7.60	7.20-7.60
	P	7.90-8.20	7.60-80	7.60-8.10	7.80-8.20
	B	7.80-8.30	7.70-8.20	7.60-8.20	7.70-8.00
EC (mS cm^{-1})	R	9.17-11.80	7.01-7.80	7.50-7.90	7.20-8.41
	P	8.00-11.31	10.40-11.12	8.12-9.50	7.20-8.74
	B	8.21-14.80	10.13-12.00	8.50-10.90	8.14-9.50
Clay (%)	R	1.28-6.90	60.65-69.17	29.60-91.00	29.01-93.10
	P	2.07-6.95	25.05-65.88	45.1-76.64	45.85-76.10
	B	2.33-8.30	51.47-68.19	30.2-66.66	51.22-77.54
Organic C (%)	R	0.95-2.15	2.34-3.22	1.83-2.25	2.14-3.19
	P	1.04-3.39	2.35-3.03	1.23-3.75	0.99-1.98
	B	0.90-4.29	3.04-3.72	2.08-4.16	1.35-2.48
Total P (mg kg^{-1})	R	567.40-552.60	1455.00-1467.00	1324.40-1336.50	1465.06-1477.67
	P	458.08-474.30	1061.00-1072.00	1434.21-1449.16	1344.30-1361.90
	B	537.30-548.12	1069.00-1980.00	1241.04-1251.96	1263.52-1291.61
Organic P (mg kg^{-1})	R	164.65-178.00	779.81-798.52	769.20-778.74	788.55-799.70
	P	53.47-62.94	75.21-87.25	89.17-100.13	71.00-74.07
	B	141.14-152.08	393.03-404.15	323.94-332.83	240.34-266.04
Inorganic P (mg kg^{-1})	R	381.16-389.49	664.81-679.04	550.19-558.38	671.15-679.84
	P	405.22-411.55	982.88-990.72	1335.10-1359.98	1272.98-1288.18
	B	390.46-399.27	1570.85-1580.80	913.12-919.87	1010.26-1024.25
Olsen P (mg kg^{-1})	R	12.55-13.11	12.38-12.97	11.32-12.39	13.05-13.99
	P	8.90-10.35	9.75-11.03	3.00-4.97	11.15-16.70
	B	7.10-8.52	9.25-13.14	13.41-14.67	9.35-10.46
Fe (mg kg^{-1})	R	78030-78390	100200-100340	85610-85870	82240-82880
	P	74150-74360	115500-115690	99800-99810	87020-87030
	B	49300-49520	126000-126120	108200-108640	81340-81390
Mg (mg kg^{-1})	R	10450-10458	7544-7673	7179-7538	4001-4070
	P	10431-10489	7109-7600	4771-4782	5402-5465
	B	10426-10483	8593-8851	6743-6745	4914-5087
Ca (mg kg^{-1})	R	10110-10160	9520-9592	9501-9575	4123-4149
	P	10100-10186	5357-5397	4547-4565	3367-3389
	B	10212-10233	4708-4727	7909-7961	5327-5376
Zn (mg kg^{-1})	R	370-373	374 -379	305 -310	340-349
	P	311-314	301-304	303-305	323-328
	B	220-228	350-358	301-308	324-326

Note*= R- Rhizosphere, B- bulk, P-pneumatophore

Table 3: Distribution of Phosphate-solubilizing bacteria (cfu × 10^3 g^{-1}) in Pikovskaya's agar and NBRIP medium

		Sediment type			Mean
	Site	Bulk	Pneumatophore	Rhizosphere	
Pikovskaya agar	Alibag	0.89	3.56	3.72	2.72^{ab}
	Versova	0.47	2.88	6.07	3.14^{bc}
	Gorai	0.20	2.97	2.80	1.99^{a}
	Mahul	1.54	3.69	5.94	3.73^{c}
	Mean	0.78^{a}	3.28^{b}	4.63^{c}	2.90
NBRIP media	Alibag	10.5	0.93	6.30	5.91^{b}
	Mahul	0.75	8.33	6.22	5.10^{ab}
	Gorai	0.61	6.68	3.92	3.74^{a}
	Versova	1.02	6.89	11.45	6.45^{b}
	Mean	3.22^{a}	5.71^{b}	6.97^{c}	5.30

3.3. Enzymatic Characterization of Phosphate-solubilizing bacteria

The phosphate-solubilizing activity ranged from 1.6±0.3 to 85.8± 0.0 µg P released per 10^8 cfu in 72 hrs of which *B. subtilis* subsp. *spizizenii* TU-B-10 showed significantly higher activity and *B. mojavensis* strain MTP16 showed significantly lower activity. It was observed that most of PSB were of rhizospheric origin (Table 4).

Table 4: Distribution of phosphate-solubilizing bacteria in different sediment types and their solubilizing activity (Mean±SD; µg P per 10^8 cfu in 72 hrs)

Name	Similarity	Site	Sediment	Activity
Bacillus atrophaeus strain F2	97%	Alibag	Pneumatophore	30.4±0.0^b
B. subtilis strain MSI-9	96%	Gorai	Rhizosphere	42.1±0.0^c
B. subtilis strain MJP1	98%	Versova	Rhizosphere	70.9±0.0^f
B. pumilus strain VB6	97%	Mahul	Rhizosphere	61.9±0.0^e
B. tequilensis strain CRRI-HN-4	97%	Mahul	Rhizosphere	73.0±0.0^g
B. mojavensis strain MTP16	97%	Alibag	Rhizosphere	1.6±0.3^a
Bacillus sp. D04-1	98%	Alibag	Pneumatophore	47.7±0.0^d
B. subtilis subsp. *spizizenii* TU-B-10	99%	Gorai	Rhizosphere	85.8±0.0^h

Note*: Values with different letters in superscript differ significantly at p = 0.05

4. Discussion

From the present study, the phosphate-solubilizers ranged from 0.47-6.07×10^3 cfu g^{-1} and 0.75-11.45×10^3 cfu g^{-1} in Pikovskaya media and NBRIP medium, respectively (Table 3). The results indicated that the rhizospheric sediment showed higher biological activity. Similarly, Kathiresan and Ravikumar (1995) [6] observed maximum species diversity in the roots and rhizospheric sediment of mangrove plant species. This is due to the secretions of carbohydrates and amino acids from root exudates of plants that enhance the growth and multiplication of bacterial species. The physicochemical and biological characteristics of rhizospheric sediment are significantly differing from bulk sediment [4] also supports the present study.

The organic anions secreted from plant roots increases the P availability compounds by desorbing inorganic P from a mineral surface and chelating or complexing cations such as Al, Fe and Ca which gets bound to P [17]. Such results were observed in the present study that rhizospheric sediment had higher load of PSB with low pH, inorganic P, iron content and higher Ca, Mg and olsen P. The pneumatophoric sediment had lower organic P. All these clearly indicate the P solubilization in and around rhizosphere compared to bulk and pneumatophoric sediment. Higher metal content in all sediment types might be due to anthropogenic input and also the natural deposition of metals in the earth crust. Alibag sediment had less iron than other sites. Inorganic P content of Alibag was lower than that of other sites. The lower inorganic P coincides with lower iron content and higher calcium and magnesium content in Alibag sediment and rhizospheric sediment than that of other sites and sediment types. Probably the low iron content would have resulted in low inorganic P in Alibag sediment and rhizospheric sediment.

In the present study, the reliability of halos developed in PVK media were not efficient compared to NBRIP media (Fig 2), many isolates showed less halo even then, they were having higher solubilizing activity and more halo in NBRIP medium plates. It is also reported that NBRIP medium is more efficient than the PVK medium for screening PSB [11]. hence, it is proposed that microbes may be screened in NBRIP assay for the identification of the most efficient phosphate-solubilizers.

In this study, from the eight isolates notably, *B. pumilus*, *B. tequilensis,* and *B. mojavensis* were observed apart from *B. subtilis* and *B. Atrophaeus*. Similar study was conducted from rhizospheric mangrove sediment of Laguna de Balandra, Mexico where the PSB's isolated were *B. atrophaeus, B. amyloliquefaciens, Vibrio proteolyticus, Paenibacillus macerans,* and *Xanthobacter agilis* [20]. *Bacillus* species have been reported as biocontrol agents effective against numerous root pathogens [10] and is the most abundant genus in the rhizosphere [14]. Three species of phosphobacteria, belonging to the same genus (*Bacillus*) was isolated from Pichavaram mangrove [15]. Hence from the present study, the abundance of Bacillus sp as PSB in mangrove sediments coincides with previous studies.

It was observed that most of PSB were isolated from rhizospheric sediment and first time reporting the inhabitant nature of PSB in rhizospheric zone with clear indication of solubilization due to low pH and lower inorganic P in rhizospheric sediment. This was also supported by other physicochemical parameters such as high organic P, Olsen P, and higher bacterial count along with low inorganic P.

The solubilizing activity ranged from 1.6±0.3 to 85.8± 0.0 µg P per 10^8 cfu in 72 hrs. *B. subtilis* subsp. *spizizenii* TU-B-10 showed significantly higher activity (85.8±0.0 µg P released per 10^8 cfu in 72 hrs). *B. mojavensis* strain MTP16 showed significantly lower activity (1.6±0.3 µg P released per 10^8 cfu in 72 hrs). Similar observation reports that B. *brevis* showing solubilization activity of 7.5 µg P ml^{-1} and *B. thuringiensis* with the activity of 20.0 µg P ml^{-1} [2]. The highest reported phosphate solubilization was by an unidentified marine bacterium, 300 mg l^{-1}, isolated from the rhizosphere of the sea grass, *Zostera marina* [1]. Phosphate-solubilizing activity of *B. amyloliquefaciens* as 400 mg of phosphate per litre of bacterial suspension (10^8 cfu ml^{-1}) was reported [21]. The isolate *B. subtilis* sub. spizizenii TU-B-10 from the present study showed lower solubilizing activity than that reported in Laguna de Balandra, Mexico [21], but higher than that reported by de Freitas *et al.* (1997) [2]. Thus PSB from *A. marina* mangrove sediment with high solubilization activity can be isolated and characterized on extensive scale in order to pave a way for developing environment friendly bio-fertilizers which reduces the requirement of phosphatic fertilizers in salt affected agricultural land, aquacultural practices, mangrove afforestation programmes, bioremediation of excessively eutrophic water bodies, in molecular biology and as biosensors.

5. Conclusion

The present study revealed that higher abundance of phosphate-solubilizing bacteria was found in the rhizospheric sediment indicates the inhabitant nature of PSB in this zone with clear indication of low pH in the rhizospheric sediment.

Lower inorganic P was found in rhizospheric sediment indicating the solubilization supported by various physicochemical parameters of sediment.

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7. References

1. Craven PA, Hayasaka SS. Inorganic phosphate solubilization by rhizosphere bacteria in a *Zostera marina* community. Canadian Journal of Microbiology. 1982; 28:605-610.
2. de Freitas JR, Banerjee MR, Germida JJ. Phosphorus-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus L.*). Biology and Fertility of Soils. 1997; 24:358-364.
3. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Applied Environmental Microbiology. 2008; 74:2461-2470.
4. Gregory PJ, Hinsinger P. New approaches to studying chemical and physical changes in the rhizosphere: an overview. Plant Soil. 1999; 211:1-9.
5. Kannapiran E, Ravindran J. Dynamics and Diversity of phosphate mineralizing bacteria in the coral reefs of Gulf of Mannar. Journal of Basic Microbiology. 2012; 52:91-98.
6. Kathiresan K, Ravikumar S. Influence of tannins, sugars and amino acids on bacterial load of marine halophytes. *Environ. Ecol*. 1995; 13(1):94-96.
7. Kim KY, Jordan D, McDonald GA. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biology and Fertility of Soils. 1998; 26:79-87
8. Kuo S. Phosphorus. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (Eds.), Methods of Soil Analysis: Part 3, Soil Science Society of America Book Series (3rd ed.), No. 5, Soil Science Society of America and American Society of Agronomy, Madison, Wisconsin, USA, 1996, 869-919.
9. Muthuvel P, Udayasoorian C. Soil, plant, water and agrochemical analysis. Tamilnadu Agriculture Univ., Coimbatore, 1999, 301.
10. Nagorska K, Bikowski M, Obuchowskji M. Multicellular behaviour and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. Acta Biochimica Polonica. 2007; 54:495-508.
11. Nautiyal CS. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett. 1999; 170:265-270.
12. Olsen S, Cole C, Watanabe F, Dean L. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular Nr 939, US Gov.Print. Office, Washington, D.C, 1954.
13. Orhan I, Kupeli E, Aslan M, Kartal M, Yesilada E. Bioassay-Guided Evaluation Of Anti-Inflammatory And Antinociceptive Activities Of Pistachio, *Pistacia Vera* L. Journal of Ethnopharmacology. 2006; 105:235-240.
14. Probanza A, Lucas GJA, Ruiz PM, Ramos B, Gutierrez MFJ. Pinus pinea L. seedling growth and bacterial rhizosphere structure after inoculation with PGPR *Bacillus (B. licheniformis* CECT 5106 and *B. pumilus* CECT 5105). Applied Soil Ecology. 2002; 20:75-84.
15. Ravikumar S, Ramanathan G, Suba N, Jeyaseeli L, Sukumaran M. Quantification of halophilic Azospirillum from mangroves. Ind. J. Mar. Sci. 2002; 31(2):157-160
16. Rutzler K, Feller IC. Caribbean mangrove swamps. Scientific American. 1996; 274:94-99.
17. Ryan PR, Delhaize E, Jones DL. Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Physiol. Plant Mol. Biol., 2001; 52:527-560
18. Snedaker SC. Mangrove species zonation: why? In: Sen, D.N. and Rajpurohit, K.S. (Eds.) Contribution to the ecology of halophytes. Kluwer Academic: Netherlands. 1982, 111-125.
19. Stevenson FJ, Cole MA. Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfer, Micronurients. Second Edition. John Wiley and Sons Inc. New York, 1999.
20. Vazquez P, Holguin G, Puente ME, Lopez-Cortes A, Bashan Y. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biology and Fertility of Soils. 2000; 30:460-468.
21. Walkley JA, Black JA. Estimation of organic carbon by the chromic acid titration method. Soil Science. 1934; 1934; 37:29-31.