Journal of Academia and Industrial Research (JAIR)
Volume 3, Issue 8 January 2015

ISSN: 2278-5213

RESEARCH ARTICLE

Diversity Status of Beneficial Microflora in Saline Soils of Tamil Nadu and Pudhucherry in Southern India

V. Mohan* and Sangeetha Menon
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore-641002, TN, India
mohan@icfre.org*; +91 9443426214

Abstract

Alleviating plant salt stress and remediating saline soils are of great economic interest. Beneficial microbes such as Arbuscular Mycorrhizal (AM) fungi, Plant Growth Promoting Rhizobacteria (PGPRs), etc. are associated with many plants including trees. These beneficial microbes can cope up with salinity and help the plants to survive in such soils. In this study, diversity status of beneficial microflora from the samples collected from different salt affected areas in Tamil Nadu and Pudhucherry, South India was investigated. Total of 51 PGPR isolates (Phosphate Solubilizing Bacteria-18; *Azotobacter* spp.-16 and *Azospirillum* spp.-17) and 25 different AM fungi were made. Screened efficient PGPR isolates for plant growth hormone (IAA) production and phosphate solubilization under *in vitro*. Highest amount of IAA was produced by *Azospirillum lipoferum* (74 µg/mL) and *Azotobacter chroococcum* (36 µg/mL). Phosphate solubilizing bacterial isolates showed a high rate of phosphate solubilization efficiency (SE) with *Bacillus megaterium* indicating a SE of 140% and *Bacillus subtilis*, a SE of 120%, making both the isolates as strong phosphate solubilizers.

Keywords: Arbuscular Mycorrhizal fungi, plant growth promoting rhizobacteria, microflora, phosphate solubilizers**.**

Introduction

Salinity affects more than 7% of the Earth's land area (Parida and Das, 2005). Most of this salinity is natural but the extent of saline soils is increasing in a significant proportion of cultivated agriculture lands because of land clearing or irrigation (Munns, 2005). Salinity is considered one of the most significant environmental factors limiting plant growth, productivity (Tian *et al.,* 2004) and survival of glycophytes (Munns, 2005). Since, salinity problems are commonly associated with agricultural areas, salt resistance of tree species has received relatively little attention. However, salinity can also be of major concern in natural boreal ecosystems (Purdy *et al.,* 2005) as well as in urban areas (Lait *et al*., 2001) and industrial reclamation sites (Renault *et al.,* 2001). Salt adversely affects plants by inducing osmotic imbalance, ionic toxicity, nutrient deficiencies or a combination of the above factors (Shannon, 1977). It also upsets water balance by interfering with the activity of water channel proteins (aquaporins) which results in an increase in water flow resistance of the root system (Carvajal *et al.*, 2000; Martinez-Ballesta *et al*., 2000; Apostol *et al.,* 2002; Lopez-Berenguer *et al.,* 2006). To overcome salt-stress problems, it is possible to select salt-tolerant plants, use biological processes such as mycorrhizal or beneficial microbial interactions or desalinate soil by leaching excessive salts (Munns, 2005). The desalination of soils is not economically viable for sustainable agriculture.

Plant Growth Promoting Rhizobacteria (PGPR) is a group of bacteria that actively colonize plant roots and increase plant growth and yield. The mechanisms by which PGPR promote plant growth, include the ability to produce phytohormones, asymbiotic N_2 fixation against phyto-pathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds and also solubilization of mineral phosphates and other nutrients (Gholami *et al.,* 2009). The rhizobacteria play an important role within the interaction between soil and plants. As plants grow on marginal soils such as saline soils, the importance of the rhizobacteria increases as they mobilize nutrients and provide tolerance ability to the plants. Research on the rhizosphere beneficial microflora in saline environment finds its direct application in the exploitation of those novel beneficial microbes for phytoremediation of saline soils (Paul and Sudha, 2008; Upadhyay *et al.,* 2009). The mycorrhizal fungi improve seedling growth and survival by enhancing the uptake of nutrients and water and increasing root life span. They also help to protect the roots against other pathogenic organisms and against environmental stresses such as heavy metal toxicity or soil salinity etc. Plant-microbe interaction is gaining more attention of agronomists and microbiologists in an area of applied research and halotolerant or halophilic bacteria are being utilized in the field and they can flourish in hyper saline conditions and interacting with plants may restore these lands to productivity.

Salt tolerant beneficial microbes from different sources i.e., saline soils, rhizosphere, rhizoplane and phylloplane of different plants growing in saline areas have been isolated (Yasmin and Hasnian, 1997). These microbes were able to resist high levels of sodium chloride stress. The bacteria from different sources promote seedling growth under saline conditions (Siddique, 1887). The present study was undertaken to ascertain the diversity status of different beneficial microbes from different plantations in salt affected areas including coastal soils in Tamil Nadu and Pudhucherry (South India).

Materials and methods

Collection of soil samples: Rhizosphere soil samples were collected under the root zone of different plants in selected salt affected study sites including coastal areas of Tamil Nadu and Pudhucherry in zip lock poly bags, sealed tightly and immediately transported to laboratory. The samples were kept in refrigerator at 4ºC until further use.

Physico-chemical parameters of soil samples: Soil samples were analyzed for their physico-chemical parameters such as pH, Electrical Conductivity (EC), Available Nitrogen (N), Phosphorus (P) and Potassium (K) and micro elements such as Copper (Cu), Zinc (Zn), Magnesium (Mg) and Manganese (Mn) in Soil and Water Testing Laboratory of Institute of Forest Genetics and Tree Breeding, Coimbatore by adopting standard techniques.

Isolation and identification of PGPR from saline soil samples: Serial dilution and plating techniques as described by Parkinson *et al*. (1971) and Subba Rao (1993) were adopted for enumerating the status of Plant Growth Promoting Rhizobacteria (PGPR). Among different PGPRs, *Azotobacter* colonies were selected based on the appearance of mucoid, transparent, gummy colonies; *Azospirillum* colonies appeared as scarlet pink, round colonies and Phosphate Solubilizing Bacteria (PSB) colonies were identified based on the halo zone formed around the colonies. Population density of these PGPR organisms was also determined for each sample as CFU/g (Colony forming units/g of soil (Rodriguez-Caceras, 1982; Subba Rao, 1993). All the isolates of PGPR viz., *Azotobacter, Azospirillum* and PSB were maintained in nutrient agar slants at 4ºC for further studies. All the PGPR isolates were identified up to species level based on the following growth characteristics, staining reactions and biochemical tests (Martin *et al*., 2006).

Determination of Indole Acetic Acid (IAA) production by PGPRs: The test tubes containing nutrient broth with tryptophan (2 mg/mL) was sterilized and inoculated with 1 mL of PGPR isolates. Then the tubes were incubated for 7-8 d. After incubation, the culture broth was centrifuged at 10,000 rpm for 30 min and the pellet was discarded. About 1 mL of the supernatant was taken in a clean test tube and 2 mL of freshly prepared Salkowski's reagent (50 mL 35% $HClO_4$ + 1 mL $FeCl_3$) was added. The tubes were incubated in dark for 30 min for the development of pink colored complex. After 30 min, the absorbance was measured at 530 nm. Various concentrations of tryptophan were used as the standard. Standard graph of Indole Acetic Acid was prepared using standard values and the concentration of IAA produced by each isolate was determined by extrapolating the absorbance value in the Y-axis of the standard graph and determining the corresponding value of IAA (µg/mL) in the X-axis (Bent *et al.,* 2001).

Determination of phosphate solubilisation by PGPRs: PGPR isolates of PSB obtained were re-tested by plate assay for phosphate solubilisation in Pikovskaya's agar medium. These PSB isolates were stabbed in triplicate using sterile toothpicks. The halo zone around the colony was presumptive confirmation of phosphate solubilisation and was measured after 7 d of incubation at 30ºC. Halo size was calculated by subtracting colony diameter from the total zone of colony and halo zone. Solubilisation Efficiency (SE) was calculated by the formula as described by (Sharma *et al*., 2007).

$$\text{Solubilisation Efficiency (SE)} = \frac{\text{Solubilisation diameter}}{\text{Growth diameter}} \times 100$$

Isolation and identification of Arbuscular Mycorrhizal (AM) fungi: The Arbuscular Mycorrhizal Fungal (AMF) spores were isolated and estimated by using wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and sucrose density gradient technique (Daniels and Skipper, 1982). Root colonization by the AM fungi was done by using root clearing and staining techniques (Phillips and Hayman, 1970; Koske and Gemma, 1989) and data on percent root colonization of AM fungi was also estimated by using gridline intersect method (McGonigle *et al*., 1990). The intact and the crushed spores were examined under a compound microscope and genus and species level identification of different AM fungi isolated from the saline soil samples has been done by using standard keys (Trappe, 1982; Schenck and Perez, 1987). Spores were identified based on spore morphology and sub cellular characters and compared with original descriptions (Schenck and Perez, 1987).

Results

Physico-chemical parameters of soil samples: Data on physico-chemical properties of saline soil samples collected from 17 different study locations is presented in Tables 1 and 2. It was found that the pH was found maximum (9.5) in saline soil with *Eucalyptus* trees at Kattugudalur (Acchirapakkam), followed by the sample locations of outside *Casuarina equisetifolia* plantation (8.8) at Kalapet (Pudhucherry) and saline soil with *Prosopis juliflora* trees (8.8) at Semmaru, Sathanur (Villupuram).

Table 1. Physico-chemical parameters of soil samples.

S. No.	Physico-chemical parameters	Soil sample locations								
		1	2	3	4	5	6	7	8	9
1.	pH	8.2	6.8	8.8	8.3	8.8	6.9.	8.2	7.0	6.7
2.	Electrical conductivity (dSm^{-1})	0.17	0.24	0.55	0.25	0.25	0.12	0.11	5.40	0.10
3.	Organic carbon	0.43	0.44	0.49	0.22	0.22	0.31	0.67	0.39	0.38
4.	Soil texture	Sandy loam	Loamy sand	Sandy loam	Loamy sand	Loamy sand	Sand	Sand	Loamy sand	Sand
5.	Available Nitrogen (Kg ha^{-1})	120.65	103.13	138.68	71.83	64.90	60.3	30.33	156.88	58.10
6.	Phosphorus (Kg ha^{-1})	16.12	16.32	13.78	16.87	19.54	17.51	19.72	20.23	19.57
7.	Potassium (ppm)	33.45	5.56	31.23	9.97	14,89	5.01	7.60	41.44	8.97
8.	Bulk density (gm/cc)	1.51	1.60	1.45	1.32	1.22	1.07	1.67	1.32	1.46
9.	Sodium (ppm)	NA	22.77	599.0	NA	609.14	48.4	42.65	484.55	62.92
10.	Calcium (meq/100 g)	0.23	0.12	0.11	0.44	0.21	NA	0.13	0.16	0.53
11.	Magnesium (meq/100 g)	0.15	0.14	0.15	0.31	0.21	NA	0.13	0.19	0.19
12.	Copper (ppm)	1.9	1.5	1.4	1.2	1.7	NA	1.5	2.32	2.4
13.	Zinc (ppm)	1.6	1.7	1.9	1.3	2.0	NA	1.0	1.9	0.32
14.	Manganese (ppm)	3.9	5.2	5.7	2.8	2.5	NA	5.6	543	6.3
15.	Iron (ppm)	27.8	25.7	41.7	51.4	19.9	NA	25.7	29.7	7.3

NA: Not available, 1. Saline soil paddy field, Semmaru 1, Sathanur (Villupuram), 2. Saline soil with *Prosopsis juliflora* Semmaru-3, Sathanur (Villupuram), 3. Inside *Casuarina equisetifolia* plantation, Kalapet-1 (Puducherry), 4. Outside, *Casuarina equisetifolia* plantation, Kalapet-2 (Puducherry), 5. Coastal soil, Kalapet-3 (Puducherry), 6. *Casuairna equisetifolia* Plantation, 7. Sonangkuppam-1 (Cuddalore), 8. Salt affected soil, Marakanam, 9. Outside *Casuairna equisetifolia* Plantation Sonangkuppam-2(Cuddalore).

Table 2. Physico-chemical parameters of soil samples.

S. No.	Physico-chemical parameters	Soil sample locations							
		10	11	12	13	14	15	16	17
1.	pH	5.9	5.8	9.5	7.5	7.0	7.3	7.3	7.9
2.	Electrical conductivity (dSm^{-1})	0.12	0.13	0.81	0.13	0.15	1.4	4.6	4.3
3.	Organic carbon	1.03	0.59	0.57	0.29	0.35	0.8	0.5	1.0
4.	Soil texture	Sand	Loamy sand	Loamy sand	Loamy sand	Sand	Sandy loam	Sandy loam	Loamy sand
5.	Available Nitrogen (Kg ha^{-1})	60.55	117.83	42.90	116.65	62.23	257.8	268.2	232.2
6.	Phosphorus (Kg ha^{-1})	19.83	19.14	20.18	20.73	19.71	21.3	24.5	24.4
7.	Potassium (ppm)	8.97	6.60	5.89	7.63	6.13	195.8	139.9	68.9
8.	Bulk density (gm/cc)	1.46	1.31	1.32	1.35	1.47	1.4	1.3	1.3
9.	Sodium (ppm)	47.35	23.66	676.9	28.42	50.4	691	690.5	190.2
10.	Calcium (meq/100 g)	NA	0.24	NA	0.19	NA	0.24	0.24	0.32
11.	Magnesium (meq/100 g)	NA	0.15	NA	0.21	NA	0.10	0.08	0.18
12.	Copper (ppm)	NA	1.6	NA	1.8	NA	1.7	1.8	1.3
13.	Zinc (ppm)	NA	1.5	NA	1.3	NA	0.9	1.2	1.1
14.	Manganese (ppm)	NA	4.9	NA	4.9	NA	940	5.2	4.6
15.	Iron (ppm)	NA	34.8	NA	30.6	NA	36.7	45.1	37.7

10. *Casuairna equisetifolia* Plantation, Singarathopu-1 (Cuddalore), 11. Outside *Casuairna equisetifolia* Plantation, Singarathopu-2 (Cuddalore), 12. Saline soil Eucalyptus trees, Kattugudalur-1 (Acchirupakkam), 13. Saline soil (Neem and Phoenix trees), Kattugudalur-2 (Acchirupakkam), 14. Coastal soil, Singarathopu-3 (Cuddalore), 15. *Casuarina equistifolia* plantation, Moorthy palayam-1 (Karur), 16. Outside *Casuarina equisetifolia* plantation, Moorthy palayam-2 (Karur), 17. Saline soil, Ammapatty (Karur).

The pH was found minimum (5.8 and 5.9) in both inside and outside *C. equisetifolia* plantations at Singarathope (Cuddalore) respectively. The pH of other study locations varied from 6.7 to 8.2. It was also recorded that available nitrogen, phosphorus and potassium are low in all the samples collected from various study locations. The element sodium was found maximum (691 and 690.5 ppm) in sample locations of both inside and outside *C. equistifolia* plantations at Moorthy palayam (Karur), this is followed by the sample location at saline soil with *Eucalyptus* trees (676.9 ppm) at Kattugudalur (Acchirupakkam), outside *C. equisetifolia* plantation (609.14 ppm) at Kalapet (Pudhucherry), saline soil with *P. juliflora* (599 ppm) at Semmaru, Sathanur (Villupuram) and salt affected soil (484.55 ppm) at Marakanam as compared to other study locations. The micro nutrient status is found minimum in all the study locations.

Diversity status of PGPRs from different saline soil samples: Survey was undertaken and collected roots and rhizosphere soil samples in 17 different study locations in Tamil Nadu and Pudhucherry. The samples were analyzed and data on population density of PGPRs (*Azotobacter, Azospirillum* and Phosphate Solubilizing Bacteria) were recorded and presented in Tables 3 and 4. Variation in population density of PGPRs was observed in samples collected from various study locations.

Table 3. Population density of PGPR recorded from various samples collected from different salt affected sites.

S. No.	Soil sample locations	Population density of PGPR's (CFU/g)		
		PSB	*Azotobacter* sp.	*Azospirillum* sp.
1.	Saline soil paddy field, Semmaru 1, Sathanur (Villupuram)	13×10^5	12×10^5	26×10^5
2.	Saline soil outside paddy field, Semmaru 2, Sathanur (Villupuram)	20×10^5	2×10^5	3×10^5
3.	Saline soil with *Prosopsis juliflora* Semmaru-3, Sathanur (Villupuram)	22×10^5	19×10^5	40×10^5
4.	Inside *Casuarina equisetifolia* plantation, Kalapet-1 (Puducherry)	37×10^5	63×10^5	27×10^5
5.	Outside *Casuarina equisetifolia* plantation, Kalapet-2 (Puducherry)	4×10^5	5×10^5	4×10^5
6.	Coastal soil, Kalapet-3 (Puducherry)	Nil	6×10^5	9×10^5
7.	Inside *Casuairna equisetifolia* Plantation, Sonangkuppam-1 (Cuddalore)	55×10^5	22×10^5	27×10^5
8.	Salt affected soil, Marakanam	22×10^5	11×10^5	6×10^5
9.	Outside *Casuairna equisetifolia* Plantation Sonangkuppam-2(Cuddalore)	14×10^5	7×10^5	11×10^5
10.	Inside *Casuairna equisetifolia* Plantation, Singarathopu-1 (Cuddalore)	40×10^5	77×10^5	25×10^5
11.	Outside *Casuairna equisetifolia* Plantation, Singarathopu-2 (Cuddalore)	12×10^5	3×10^5	4×10^5
12.	Saline soil *Eucalyptus* trees, Kattugudalur-1 (Acchirupakkam)	Nil	17×10^5	13×10^5
13.	Saline soil (Neem and Phoenix trees), Kattugudalur-2 (Acchirupakkam)	20×10^5	49×10^5	36×10^5
14.	Coastal soil, Singarathopu-3 (Cuddalore)	12×10^5	18×10^5	14×10^5
15.	Inside *Casuarina equistifolia* plantation, Moorthy palayam-1 (Karur)	46×10^5	33×10^5	48×10^5
16.	Outside *Casuarina equisetifolia* plantation, Moorthy palayam-2 (Karur)	Nil	20×10^5	6×10^5
17.	Saline soil, Ammapatty (Karur)	3×10^5	Nil	7×10^5

* Mean of 3 replications.

Table 4. PGPR isolates obtained from saline samples of different salt affected sites in Tamil Nadu and Puducherry.

S. No.	Soil sample locations	Population density of PGPR's (CFU/g)			Total
		PSB	*Azotobacter* sp.	*Azospirillum* sp.	
1.	Saline soil paddy field, Semmaru 1, Sathanur (Villupuram)	1	1	1	3
2.	Saline soil outside paddy field, Semmaru 2, Sathanur (Villupuram)	1	1	1	3
3.	Saline soil with *Prosopsis juliflora* Semmaru-3, Sathanur (Villupuram)	2	1	1	4
4.	Inside *Casuarina equisetifolia* plantation, Kalapet-1 (Puducherry)	1	1	1	3
5.	Outside *Casuarina equisetifolia* plantation, Kalapet-2 (Puducherry)	1	1	1	3
6.	Coastal soil, Kalapet-3 (Puducherry)	Nil	1	1	2
7.	Inside *Casuairna equisetifolia* Plantation, Sonangkuppam-1 (Cuddalore)	1	1	1	3
8.	Salt affected soil, Marakanam	2	1	1	4
9.	Outside *Casuairna equisetifolia* Plantation Sonangkuppam-2(Cuddalore)	3	1	1	5
10.	Inside *Casuairna equisetifolia* Plantation, Singarathopu-1 (Cuddalore)	1	1	1	3
11.	Outside *Casuairna equisetifolia* Plantation, Singarathopu-2 (Cuddalore)	1	1	1	3
12.	Saline soil *Eucalyptus* trees, Kattugudalur-1 (Acchirupakkam)	Nil	1	1	2
13.	Saline soil (Neem and Phoenix trees), Kattugudalur-2 (Acchirupakkam)	1	1	1	3
14.	Coastal soil, Singarathopu-3 (Cuddalore)	1	1	1	3
15.	Inside *Casuarina equistifolia* plantation, Moorthy palayam-1 (Karur)	1	1	1	3
16.	Outside *Casuarina equisetifolia* plantation, Moorthy palayam-2 (Karur)	Nil	1	1	2
17.	Saline soil, Ammapatty (Karur)	1	Nil	1	2

The population density of PSB was found maximum (55 x 10^5 cfu/g) in inside *C. equisetifolia* plantation at Sonangkuppam (Cuddalore), followed by inside *C. equisetifolia* plantation (46 x 10^5 cfu/g) at Moorthy palayam (Karur), inside *C. equisetifolia* plantation (40 x 10^5 cfu/g) at Singarathopu (Cuddalore) and inside *C. equisetifolia* plantation (37 x 10^5 cfu/g) at Kalapet (Pudhucherry). Minimum PSB population was found in saline soil (3 x 10^5 cfu/g) at Ammapatty (Karur) and outside *C. equisetifolia* plantation (4 x 10^5 cfu/g) at Kalapet (Puducherry). No population of PSB was recorded from samples collected from 3 locations viz., coastal soil (Kalapet), saline soil with *Eucalptus* trees at Kattugudalur (Acchirapakkam) and outside *C. equisetifolia* plantation at Moorthipalayam (Karur).

The population density of *Azotobacter* was found maximum (77 x 10^5 cfu/g) inside *C. equisetifolia* plantation, Singarathopu (Cuddalore), followed by inside *C. equisetifolia* plantation (63 x 10^5 cfu/g) at Kalapet (Puducherry), saline soil with Neem and Phoenix trees (49 x 10^5 cfu/g) at Kattugudalur (Acchirupakkam) and inside *Casuarina equisetifolia* plantation (33 x 10^5 cfu/g) at Moorthy palayam (Karur). Minimum population density of *Azotobacter* (2 x 10^5 cfu/g) was recorded in saline soil outside paddy field at Semmaru, Sathanur (Villupuram), followed by outside *C. equisetifolia* plantation (3 x 10^5 cfu/g) at Singarathopu (Cuddalore) and no population of *Azotobacter* was recorded at saline soil collected from Ammapatty (Karur).

Table 5. Occurrence and distribution of AM fungi in various samples collected from different salt affected sites.

S. No.	Soil sample locations	% root colonization of AM fungi	Soil spore population of AM fungi
1.	Saline soil paddy field, Semmaru 1, Sathanur (Villupuram)	48	126
2.	Saline soil outside paddy field, Semmaru 2, Sathanur (Villupuram)	12	21
3.	Saline soil with *Prosopsis juliflora* Semmaru-3, Sathanur (Villupuram)	49	172
4.	Inside *Casuarina equisetifolia* plantation, Kalapet-1 (Puducherry)	74	288
5.	Outside *Casuarina equisetifolia* plantation, Kalapet-2 (Puducherry)	26	74
6.	Coastal soil, Kalapet-3 (Puducherry)	9	72
7.	Inside *Casuairna equisetifolia* Plantation, Sonangkuppam-1 (Cuddalore)	79	301
8.	Salt affected soil, Marakanam	4	8
9.	Outside *Casuairna equisetifolia* Plantation Sonangkuppam-2(Cuddalore)	32	42
10.	Inside *Casuairna equisetifolia* Plantation, Singarathopu-1 (Cuddalore)	85	344
11.	Outside *Casuairna equisetifolia* Plantation, Singarathopu-2 (Cuddalore)	46	102
12.	Saline soil *Eucalyptus* trees, Kattugudalur-1 (Acchirupakkam)	49	107
13.	Saline soil (Neem and Phoenix trees), Kattugudalur-2 (Acchirupakkam)	71	214
14.	Coastal soil, Singarathopu-3 (Cuddalore)	12	85
15.	Inside *Casuarina equistifolia* plantation, Moorthy palayam-1 (Karur)	91	373
16.	Outside *Casuarina equisetifolia* plantation, Moorthy palayam-2 (Karur)	16	82
17.	Saline soil, Ammapatty (Karur)	8	10

* Mean of 3 replications.

The population density of *Azospirillum* was found maximum (48 x 10^5 cfu/g) inside *C. equistifolia* plantation, Moorthy palayam (Karur)), followed by saline soil with *Prosopsis juliflora* (40 x 10^5 cfu/g) at Semmaru, Sathanur (Villupuram) and saline soil with Neem and Phoenix trees (36 x 10^5 cfu/g) at Kattugudalur-2 (Acchirupakkam). Minimum population density of *Azospirillum* (3 x 10^5 cfu/g) was recorded in saline soil outside paddy field at Semmaru, Sathanur (Villupuram), followed by outside *C. equisetifolia* plantation (4 x 10^5 cfu/g) at Kalapet (Pudhucherry) and outside *C. equisetifolia* plantation (4 x 10^5 cfu/g) at Singarathopu (Cuddalore). Total of 51 isolates of different PGPRs viz., PSB (18 isolates), *Azotobacter* (16 isolates) and *Azospirillum* (17 isolates) were isolated based on specific colonies formed in the respective selective media (Table 4). These colonies were further purified and maintained in nutrient slants. *Azotobacter* colonies appeared as mucoid, watery, transparent and gummy colonies. *Azospirillum* colonies appeared as scarlet pink round colonies. Formation of halo zone was observed around the colonies of PSB.

Status of AM fungi from different Saline soil samples: Survey undertaken and collected roots and rhizosphere soil samples in 17 different study locations in Tamil Nadu and Pudhucherry. Samples were analyzed and data on percent root colonization and soil spore population of Arbuscular Mycorrhizal (AM) fungi was recorded and presented in Table 5. There is a variation in percent root colonization and soil spore population of AM fungi in different samples analyzed. It was observed that maximum percent root colonization and soil spore population of AM fungi was recorded from the samples of *C. equisetifolia* plantation (91% and 373/100 g soil) at Moorthy palayam Karur), followed by samples of inside *C. equisetifolia* plantation (85% and 344/100 g soil) at Singarathopu (Cuddalore), *C. equisetifolia* plantation (79% and 301/100 g soil) at Sonangkuppam (Cuddalore), *C. equisetifolia* plantation (74% and 288/100 g soil) at Kalapet (Pudhucherry) and samples of saline soil with Neem and *Phoenix* trees (71% and 214/100 g soil) at Kattugudalur (Acchirupakkam). The other samples had less than 50% root colonization of AM fungi. It was also observed that percent root colonization and soil spore population of AM fungi were found very low from the samples collected pure saline areas.

Identification of PGPR isolates: All the PGPR isolates were most tolerant to sodium chloride salt showing higher absorbance were identified up to the genus and species level by their growth characteristics, staining reactions and biochemical tests and the data is presented in Tables 6-9. The *Azotobacter* isolates were identified as *A. chroococcum* and *A. beijernickii*. The *Azospirillum* isolates were identified as *A. amazonense* and *A. lipoferum*. The PSB isolates belonging to the genus *Bacillus* were identified as *B. subtilis* and *B. megaterium* (Table 10).

Indole acetic acid production and phospahte solubilisation by PGPR isolates: All the six selected PGPR isolates (*Azotobacter*–2 isolates; *Azospirillum*–2 isolates and PSB–2 isolates) were further studied to determine the efficacy for IAA production ability under *in vitro* and the data is shown in Table 10. All the isolates had the efficacy on IAA production but variation in quantity of growth hormone production. Highest amount of IAA was produced by *Azospirillum lipoferum* (74 µg/mL), followed by *Azotobacter chroococcum* (36 µg/mL). The PSB isolates of *B. subtilis* and *B. megaterium* produced low amount of IAA as expected since they are basically potential phosphate solubilizers. Phosphate solubilisation efficiency of PSB isolates showed a high rate of phosphate solubilization with *B. megaterium* indicating a SE of 140 and *B. subtilis* indicating SE of 120, making both the isolates as strong phosphate solubilizers (Table 11).

Table 6. Growth characteristics of *Azotobacter* isolates.

Growth characteristics	*Azotobacter* 1	*Azotobacter* 2
Growth on Jensen's medium	White mucoid colonies	White watery colonies
Temperature (ºC)	28-30	28-30
pH	8.2	8.2
Oxygen requirement	Strict aerobe	Strict aerobe
Colony size (mm)	1	1.2
Colony shape	Round	Round
Pigmentation	Black pigmentation in older culture	Pale white
Edge of the colony	Entire	Entire
Consistency of the colony	Smooth	Smooth
Elevation of the colony	undulate	undulate
Opacity of the colony	opaque	opaque

Table 7. Growth characteristics of *Azospirillum* isolates.

Growth characteristics	*Azospirillum* 1	*Azospirillum* 2
Growth on Rojo-Congo agar medium	Scarlet pink colonies	Scarlet pink colonies
Temperature (ºC)	35	41
pH	6.0	7.0
Oxygen requirement	Facultative anaeorobe	Facultative anaerobe
Colony size (mm)	1	1
Colony shape	Round	Round
Pigmentation	Yellow pigmentation in older culture	Scarlet pink
Edge of the colony	Entire	Entire
Consistency of the colony	Smooth	Smooth
Elevation of the colony	undulate	Undulate
Opacity of the colony	opaque	opaque

Table 8. Growth characteristics of Phosphate Solubilizing Bacterial (PSB) isolates.

Growth characteristics	PSB 1	PSB 2
Growth on Pikovskaya's medium	Milky white mucoid colonies with halo zone formation	Pale white mucoid colonies with halo zone formation
Temperature (ºC)	28-30	28-30
pH	7.5-8.0	7.0
Oxygen requirement	Strict aerobe	Strict aerobe
Colony size (mm)	1.2	1.2
Colony shape	Round	Round
Pigmentation	Creamy White	Creamy White
Edge of the colony	Entire	Entire
Consistency of the colony	Rough	Rough
Elevation of the colony	Undulate	undulate
Opacity of the colony	Opaque	opaque

Table 9. Staining and Biochemical characters of PGPR isolates.

Identification tests	*Azotobacter* 1	*Azotobacter* 2	*Azospirillum* 1	*Azospirillum* 2	PSB 1	PSB 2
Motility	+	+	+	+	++	+
Cell size (width x length)(µm)	1.5 x 2.5	2 x 3	1.0 x 4	0.8 x 3	1.6 x 2.4	0.8 x 1.8
Gram staining	G-ve ovoid cells	G-ve ovoid cells	G-ve curved rods	G-ve curved rods	G+ve rods in chains	G+ve rods in chains
Spore staining	-	-	-	-	+, central spores	+, central spores
Catalase	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Triple Sugar Iron (TSI) agar	K/K	K/K	-/-	-/-	A/A	A/A
Sulphur Indole Motilty (SIM)	+	+	+	+	+	+
Urea utilization	+	+	+	+	+	-
Citrate Utilization	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	+	+

(+) – Present, (-) – Absent, A-Acid production, K-Alkaline reaction.

Table 10. List of identified PGPR isolates.

S. No.	Isolate code	Sample No.	Identified species
1.	*Azotobacter* 1	11	*Azotobacter chroococum*
2.	*Azotobacter* 2	16	*Azotobacter beijernickii*
3.	*Azospirillum* 1	4	*Azospirillum amazonase*
4.	*Azospirillum* 2	14	*Azospirillum lipoferum*
5.	PSB 1	1	*Bacillus subtilis*
6.	PSB 2	15	*Bacillus megaterium*

Table 11. IAA production and phosphate solubilization by PGPR isolates.

S. No.	Isolates	IAA produced (µg/mL)	Halo size (mm)	Solubilization efficiency (SE)
1.	Azotobacter 1 (*Azotobacter chroococum)*	36.0	NA	NA
2.	Azotobacter 2 (*Azotobacter beijernickii)*	22.0	NA	NA
3.	Azospirillum 1 (*Azospirillum amazonase)*	14.0	NA	NA
4.	Azospirillum 2 (*Azospirillum lipoferum)*	74.0	NA	NA
5.	PSB 1 (*Bacillus subtilis)*	5.0	0.6	120
6.	PSB 2 (*Bacillus megaterium)*	4.0	0.7	140

NA: Not applicable.

Discussion

Physico-chemical parameters of soil samples: Physico-chemical parameters of different soil samples revealed high saline conditions as indicated by high concentrations of sodium, alkaline pH and high Electrical Conductivity. Some of the samples indicated low electrical conductivity as they were taken from areas colonized by shrubs or tress growing in the coastal and salt affected areas. The observations are in accordance with the findings made by (Massoud *et al.,* 1988) and they studied that the pH of the sodic soil is mostly above 8.5 while for saline soil it is less than 8.5. Similar results were also reported by (Kang and Wilson, 1987; Louis and Lim, 1987) in tropical soils characterized by low nutrient status. Similarly, Ritsema and Dekker (1994) have observed the variation in soil nutrient availability with space and time.

Diversity status of PGPR from different saline soil samples: A total of 51 isolates of PGPRs (18 PSB isolates; 17 *Azospirillum* isolates and 16 *Azotobacter* isolates) were isolated in 17 different study locations. The population density of these PGPRs was estimated and it was found that the samples of Coastal soil (Kalapet-3, (Pudhucherry of Sample No. 6), saline soil (*Eucalyptu*s trees, Kattugudalur-1, Acchirupakkam of sample No. 12) and outside *Casuarina equisetifolia* plantation (Moorthy Palayam-2, Karur of sample No. 16) did not record any PSB isolates. Similarly, *Azotobacter* isolates was not found in samples collected from saline soil (Ammapatty, Karur of sample No. 17). The samples collected from 17 different salt affected areas exhibited presence of different species of *Azotobacter*, *Azospirillum* and PSB. Environmental factors affected the occurrence and distribution of these PGPR organisms. Earlier studies indicated that isolation of *Azospirillum* from the rhizosphere by (Caballero-Mellado and Valdés, 1983) and also from many tropical trees (Subba Rao, 1984).

Species of *Pseudomonas* such as *P. striata, P. cissicola, P. fluorescencs, P. pinophillum, P. putida, P. aeroginosa, P. stutzeri* have been isolated from rhizosphere of brassica, chickpea, maize, soya bean and other crops, desert soils and Antartica lake and *Bacillus* viz., B*. brevis, B. cereus, B. circulans, B. flimus, B. megaterium, B. polymxa* and *B. subtilis* from rhizosphere of legumes, cereals, jute, chilli and oat by (Tilak *et al.,* 2005). Fifty strains of PSB viz., *Bacillus subtilis* were from the saline tract of Vidharba which are currently used as bio-fertilizers by local farmers (Trembeker *et al.,* 2009). IST (induced systemic resistance) to salt tolerance with *Arabidopsis* using *Bacillus subtilis* GBO3, a species that has previously been used as a commercial biological control agent by (Klopper *et al*., 2007). The genus and species level identification of these PGPRs were made based on their growth characteristics, staining reactions and biochemical tests. The *Azotobacter* isolates were identified as *Azotobacter beijernickii* and *A. chroococcum* species. The *Azospirillum* isolates were identified as *Azospirillum amazonense* and *A. lipoferum*. The PSB isolates belonging to the genus *Bacillus* was identified as *B. subtilis* and *B. megaterium*. Similar kind of studies was carried out by many researchers. *Pseudomonas pulifori* isolates were isolated from root free soil, rhizosphere and rhizoplane of wheat growing in alkaline soil and *Bacillus subtilis* isolates from the saline tract (Gaur, 2004).

Indole acetic acid production and phospahte solubilization by PGPR isolates: In the present study, all the selected PGPR isolates were further tested for IAA production and phosphate solubilisation ability under *in vitro* conditions and got positive results. Earlier researchers examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate (Goldstein, 1986).

The bacterial genera having phosphate solubilizing capacity viz., *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aereobacter, Flavobacterium* and *Erwinia* were also reported (Sperberg, 1958). There are considerable populations of phosphate-solubilizing bacteria in soil and in plant rhizospheres. A considerably higher concentration of phosphate-solubilizing bacteria is commonly found in the rhizosphere in comparison with non-rhizosphere soil. Contrary to the earliest reports, it was reported that salt sensitivity of *A. lipoferum* strains from the roots of kallar grass by (Reinhold *et al.,* 1986) suggests that osmotolerance in *A. lipoferum* may also depend on the source of soil or plant. The other studies, Reinhold *et al.* (1986) and Bilal *et al.* (1990) have pointed out that occurrence of three species of *Azospirillum* viz., *A. brasilense, A. lipoferum, A. halopraferens* in the rhizosphere and rhizoplane on Kallar grass, grown as pioneer in high salinity soils indicate that these isolates should be salt tolerant. The IAA producing PGPR have been isolated from Kallar grass (*Leptochloa fusca* (L.) Kunth) grown in salt affected soil of Pakistan by (Perrig *et al.,* 2007) and their growth promoting effects have been documented on rice (Mirza *et al.,* 2006). Total of 150 bacterial isolates belonging to *Bacillus, Pseudomonas, Azotobacter* and *Rhizobium* from different rhizosphere soils of chick pea in the vicinity of Allahabad was recorded by Joseph *et al.* (2007). These test isolates were biochemically characterized and screened *in vitro* for their plant growth promoting traits like production of Indole Acetic Acid (IAA), ammonia (NH_3), hydrogen cyanide (HCN), siderophore and catalase and (Guang-Can *et al.,* 2008) have screened and isolated inorganic P-solubilizing bacteria (IPSB) and organic P-mineralizing bacteria (OPMB) in soils taken from subtropical flooded and temperate non-flooded soils and compared inorganic P-solubilizing and organic P-solubilizing abilities between IPSB and OPMB. Ten OPMB strains were isolated and identified as *Bacillus cereus* and *B. megaterium*, and five IPSB strains as *B. megaterium*, *Burkholderia caryophylli*, *Pseudomonas cichorii* and *P. syringae*. A total of 72 bacterial isolates belonging to *Azotobacter*, fluorescent *Pseudomonas*, *Mesorhizobium* and *Bacillus* were isolated from different rhizospheric soil and plant root nodules in the vicinity of Aligarh (Farah *et al.,* 2008). These test isolates were biochemically characterized. These isolates were screened *in vitro* for their plant growth promoting traits like production of Indole Acetic Acid (IAA), ammonia (NH_3), hydrogen cyanide (HCN), siderophore, phosphate solubilization and antifungal activities. A total of 20 isolates of bacteria (*Azotobacter* sp*., Acinetobacter* sp*., Bacillus* sp*., Citrobacter* sp*., Flavobacterium* sp*., Klebsiella* sp*., Nitrosomonas* sp*., Pseudomonas* sp*., Rhizobium* sp*., Thiobacillus* sp*.*, *Azospirillum* sp*., Azotobacter chrococcum, Bacillus panthothenticus, Chromobacterium violaceum, C. lividum, Escherichia coli, Flavobacterium breve, Klebsiella aerogenes, Spaerotillus natans* and *Staphylococcus epidermidis*)*;* nine isolates of fungi (*Aspergillus niger*, *Bisporomyces* sp*., Monilia* sp*.*, *Cephalosporium* sp*.*, *Verticillum* sp*., Gliocladium* sp*., Penicillium* sp*., Nelicocephalum* sp*.* and *Cunninghamella* sp*.*) and seven isolates of Actinomycetes (*Streptomyces, Streptosporangium, Nocardia, Thermomonospora, Thermoactinomyces, Micromonospora, Mycobacterium)* from Rhizosphere at Wamena Biological Garden, Jayawijaya, Papua (Widawati *et al.,* 2005).

Conclusion

Diversity status of beneficial microorganisms such as Plant Growth Promoting Rhizobacteria (PGPR: Phosphate Solubilizing Bacteria–18; *Azotobacter* spp.–16 and *Azospirillum* spp.–17) and Arbuscular Mycorrhizal (AM) fungi (25 different species) from 17 different salt affected sites in Tamil Nadu and Pudhucherry was investigated. Screened efficient PGPR isolates for phytohormone (IAA) production and phosphate solubilization under *in vitro*. The PGPR isolates *viz., Azospirillum lipoferum* and *Azotobacter chroococcum* produced high amount of IAA (74 µg/mL and 36 µg/mL respectively) as compared to other isolates. The phosphate solubilizing bacterial isolates of *Bacillus megaterium* showed a high rate (140%) of phosphate solubilization efficiency followed by *Bacillus subtilis* (120%), making both the isolates as strong phosphate solubilizers. Exploitation of these beneficial microbes as bio-inoculants for production of quality tree seedlings in nurseries and out planting performance of these saplings in various salt affected areas of the country should be undertaken.

Acknowledgements

Authors are highly grateful to the Director, IFGTB, Coimbatore, for providing all the facilities and encouragement.

References

1. Apostol, K.G., Zwiaze, J.J. and Mackinnon, M.D. 2002. NaCl and Na_2SO_4 alter responses of jack pine (*Pinus banksiana*) seedlings to boron. *Pl. Soil*. 240: 321-329.
2. Bent, E., Tuzun, S., Chanway, C.P. and Enebak, S. 2001. Alterations in plant growth and in root hormone levels of lodge pole pines inoculated with rhizobacteria. *Can. J. Microbiol.* 47: 793-800.
3. Bilal, R., Ghulam, R., Querishi, J.A. and Malik, K.A. 1990. Characterization of *Azospirillum* and related diazotrophs associated with roots of plants growing in saline soils. *World J. Microbiol. Biotech*. 6: 46-52.
4. Caballero-Mellado, J. and Valdés, M. 1983. Incidencia de *Azospirillum* en algunas gramíneas del trópico subhúmedo cálido de México. *Turrialba.* 33: 83-88.
5. Carvajal, M., Cerda, A. and Martinez, V. 2000. Does calcium ameliorate the negative effect of NaCl on melon root water transport by regulating aquaporin activity? *New Phytol*. 145: 439-448.
6. Daniels, B.A. and Skipper. H.D. 1982. Methods for recovery and quantitative estimation of propagules from soil. In: Schenck, N.C. editor. *Methods and Principles of Mycorrhizal Research*, 29-36: American Phytopathological Society: St. Paul. p.244.
7. Farah, A., Iqbal, A. and Khan, M.S. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res.* 163: 173-181.

8. Gaur, S. 2004. Diacteylphoroglucinol-producing *Pseudomonas* does not influence AM fungi in wheat rhizosphere. *Curr. Sci*. 86: 453-457.
9. Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet-sieving and decanting. *Trans. Br. Mycol. Soc.* 46: 235-244.
10. Gholami, A., Shahsavani, S. and Nezarat, S. 2009. The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *World Acad. Sci. Engg. Technol.* 49: 19-24.
11. Goldstein, A.H. 1986. Bacterial solubilization of mineral phosphates: Historical perspective and future prospects. *Am. J. Altern. Agri*. 1: 51-57.
12. Guang-Can, T.A.O., Shu-Jun, T.I.A.N., Miao-Ying, C.A.I. and Guang-Hui, X.I.E. 2008. Phosphate solubilizing and mineralizing abilities of bacteria isolated from soils. *Pedosphere.* 18(4): 515-523.
13. Joseph, B., Ranjan, R.R. and Lawrence, R. 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *Int. J. Pl. Prod.* 1(2): 141-151.
14. Kang, B.T. and Willson, G.F. 1987. The development of alley forming as a promising agro-forestry technology. *In:* Steppler, H.A. and Nair, P.K.R editors. *Agroforestry A decade of development*. ICRF Nairobi- Kenya, pp.227-243.
15. Klopper, J.W., Gutierrez, A. and Mcinroy, J.A. 2007. Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth promoting rhizobacteria. *Can. J. Microbiol.* 53: 159-167.
16. Koske, R.E. and Gemma, J.N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycol. Res*. 92: 486-488.
17. Lait, C.G., Saelim, S., Zwiazek, J.J. and Zheng, Y. 2001. Effect of basement pump effluent on the growth and physiology of urban black ash and green ash ornamental trees. *J. Arboriculture*. 27: 69-77.
18. Lopez-Berenguer, C., Garcia-Vaguer, C. and Carvajal, M. 2006. Are root hydraulic conductivity responses to salinity controlled by aqua porins in broccoli plants? *Pl. Soil*. 279: 13-23.
19. Louis, I. and Lim, G. 1987. Spore density and root colonization of vesicular-arbuscular mycorrhizas in tropical soil. *Trans. Br. Mycol. Soc*. 88: 207-212.
20. Martin, D., Stanley, F., Eugene, R., Karl-Heinz, S and Erok, S. 2006. *The Prokaryotes: A handbook on the biology of bacteria,* 3rd ed. Vol-I-VII.
21. Martinez-Ballesta, M.D., Martinez, V. and Carvajal, M. 2000. Regulation of water channel activity in whole roots and in protoplasts from roots of melon plants grown under saline conditions. *Aust. J. Pl. Physiol***.** 27: 685-691.
22. Massoud, F.I., Abrol, P. and Jain, S.P.Y. 1988. Salt-Affected Soils and their Management. *FAO Soil. Bull.* 39: 3-10.
23. McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. and Swan, J.A. 1990. A method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytol.* 115: 495-501.
24. Mirza, M.S., Mehnaz, S., Normand, P., Combaret, C/P., Loccoz, Y.M., Balley, R. and Malik, K.A. 2006. Molecular characterization and PCR detection of a nitrogen fixing *Pseudomonas* strain promoting rice growth. *Biol. Fertil. Soil.* 43: 163-170.
25. Munns, R. 2005. Genes and salt tolerance: bringing them together. *New Phytol*. 167: 645-663.
26. Parida, A.K. and Das, A.B. 2005. Salt tolerance and salinity effect on plants: A review. *Ecotoxicol Environ. Saf*. 60: 324-349.
27. Parkinson, D.J., Gray, R. and Williams, S.T. 1971. Methods of studying the ecology of soil micro-organisms. Oxford, Blackwell Scientific publications, p.116.
28. Paul, D. and Sudha, N. 2008. Stress adaptations in a Plant Growth Promoting Rhizobacterium (PGPR) with increasing salinity in the coastal agricultural. *J. Basic Microbiol.* 48: 378-384.
29. Perrig, D., Boiero, M.L., Masciarelli, O., Penna, C., Ruiz, O.A., Cassan, F. and Luna, V. 2007. Plant growth promoting compounds produced by two strains of *Azospirillum brasilense* and implications for inoculant formation. *Appl. Microbiol. Biotechnol*. 75: 1143-1150.
30. Phillips, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55: 158-161.
31. Purdy, P.G., Macdonald, S.E., Lieffers, V.J. 2005. Naturally saline boreal communities as models for reclamation of saline oil sand tailings. *Restor. Ecol*. 13: 667-677.
32. Reinhold, B., Thomas, H., Ernst-Georg, N. and Istvan, F. 1986. Close association of *Azospirillum* and Diazotrophic rods with different root zones of Kallar grass. *Appl. Environ. Microbiol.* 52: 520.
33. Renault, S., Croser, C., Franklin, J.A. and Zwiazek, J.J. 2001. Effects of NaCl and Na_2SO_4 on red-osier dogwood (*Cornus stolonifea* Michx) seedlings. *Pl. Soil*. 233: 261-268.
34. Ritsema, C.J. and Dekker, L.W. 1994. How water moves in a water repellent sandy soil 2. Dynamics of fingered flow. *Water Resour. Res***.** 30: 2519-2531.
35. Rodriguez-Caceras, A. 1982. Improved medium for isolation of *Azospirillum* sp. *AEM.* 44(4): 990-991.
36. Schenck, N.C and Perez, Y 1987. Manual for the Identification of VA mycorrhizal fungi. Synergistic publications, Gainesville, Florida, p.286.
37. Shannon, M.C. 1977. Adaptation of plants to salinity. *Adv. Agron*. 60: 76-120.
38. Sharma, K., Dak, G., Agrawal, A., Bhatnagar, M. and Sharma, R. 2007. Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and seedling growth. *J. Herbal Med. Toxicol.* 1(1): 61-63.
39. Siddique, S. 1997. Growth effects of *Triticum aestivum* seedling under NaCl stress after inoculating with plasmid free bacterial strains. *Endevours in Biotechnol.,* pp.97-112.
40. Sperberg, J.I. 1958. The incidence of apatite-solubilizing organisms in the rhizosphere and soil. *Aust. J. Agric. Res*. 9: 778.
41. Subba Rao, N.S. 1984. Phosphate solubilizing microorganisms. *In: Biofertilizers in Agriculture.* 2nd edition. Oxford and IBH Publishing Co. New Delhi. Bombay, Calcutta, India, pp.126-132.
42. Subba Rao, N.S. 1993. Biofertilizers in Agriculture and Forestry, 3rd edition. Oxford & IBH publishing Co. Pvt. Ltd, New Delhi, pp.69-131.
43. Tian, C.Y., Feng, G., Li. X.L. and Zhang, F.S. 2004. Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. *Appl. Soil Ecol*. 26: 143-148.
44. Tilak, K.V.B.R., Ranganayaki, N., Pal, K.K., De, R., Saxena, K., Nautiyal, C.S., Mittal, S., Tripathi, A.K. and Johri, B.N. 2005. Diversity of plant growth and soil health supporting bacteria. *Curr. Sci.* 89: 136-150.
45. Trappe, J.M. 1982. Synoptic key to the genera and species of Zygomycetous fungi. *Phytopathol.* 72: 1102-1108.
46. Trembeker, D.H., Gulhane, S.R., Somkuwar, D.O., Ingle, K.B., Kanchalwar, S.P., Upadhye, M.A. and Bidwai, U.A. 2009. Potential *Rhizobium* and Phosphate Solublisers as a bio-fertilizer from saline belt of Akola and Buldhana districts (India). *Res. J. Agri. Biol. Sci.* 5(4): 578-582.
47. Upadhyay, K., Singh, D.P. and Salika, R. 2009. Genetic diversity of Plant Growth Promoting Rhizobacteria iIsolated from rhizospheric soil of wheat under saline condition. *J. Curr. Microbiol.* 59: 489-496.
48. Widawati, S., Suliasih, Latupapua, H.J.D. and Sugiharto, A. 2005. Bio-diversity of Soil Microbes from Rhizosphere at Wamena Biological Garden (WBiG), Jayawijaya, *Papua. Biodiversitas*. 6(1): 6-11.
49. Yasmin, A. and Hasnian, S. 1997. Moderately halophilic bacteria associated with the roots of *Chenopdium album, Oxalis cornocula* and *Lyceum edgeworthii*. *Pak. J. Zool.* 29: 249-257.