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Effects of saline tolerant *Azospirillum* species on the growth parameters of mangrove seedlings

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Abstract

Five species of *Azospirillum* isolated from Manakkudi mangrove ecosystem were subjected for their efficiency to find out their growth parameters potential for the successful establishment of mangrove seedlings. Of the isolated five *Azospirillum species*, *Azospirillum lipoferum* (60%) was found to be the dominant one. But the level of maximum indole acetic acid (IAA) production (19.8mg.ml^{-1}) and nitrogen fixation (5.9 $C_2H_2hr^{-1}$) was identified with *A. brasilense*. Further, *A. brasilense* showed significant ($p<0.05$) level of increased growth parameters [maximum root length (29.55%), average root length (7.39%), total Chl (55.36%), carrotenoids (28.57%), Chl b (37.50%), carbohydrates (90.91%) and total amino acids (78.95%)] in *Avicennia officinals* when compared with control group. Further, *A. brasilense* also showed significant ($p<0.05$) level of increased growth parameters [average number of primary roots (40%), average biomass (44.44%), average shoot biomass (55.56%), total Chl (20%), Chl b (77.78%) and carotenoid (1.54%)] in *C. decandra* seedlings when compared with control group. Similarly, the average number of primary roots (23.08%), average root biomass (15.52%), average shoot biomass (15.30%), carbohydrate (20%) and total amino acids (44.44%) were found significant ($p<0.05$) in *A. irakense* inoculated *R. apiculata* seedlings. In conclusion, *Azospirillum brasilense* was found better for the growth of *Avicennia officinalis* and *Ceriops decandra* seedlings, but *Azospirillum irakense* was found better for *Rhizophora apiculata* seedlings.

Key words

Azospirillum, Indole acetic acid, Mangroves, Nitrogen fixation

Introduction

Mangroves are tropical trees being deforested at an alarming scale (Kathiresan and Bingham, 2001) and hence it is imperative to consider strategies for their preservation and reforestation. Especially in semi-arid regions of the world where mangroves do not efficiently regenerate themselves, artificial reforestation might be a possible solution. Moreover, global warming and sea level rise recently affects the establishment of mangrove seedlings in marine soil due to the poor microbial counts, low level of important nutrients and high soil salinity (Kathiresan and Bingham, 2001). In general, numerous microorganisms are proved to have positive growth promotion constitution (Schenk, 2006; Stockdale and Brookes, 2006). Among them, *Azospirillum* are nitrogen-fixing organisms that live in close association with plants in the rhizosphere. *Azospirillum* plant association leads to the enhanced development and yield of different plants under appropriate growth conditions (Steenhoudt and Vanderleyden, 2000; Bano *et al.*, 1997). Inoculation of *Azospirillum* can alter the root morphology which can ascribe to the bacterial production of plant growth regulating

substances (Umali *et al*., 1980). Marine *Azospirillium* is receiving much attention due to its ability to tolerate high salt, nitrogen fixing property, enhancing mineral uptake production of growth promoting substances (Ravikumar *et al*., 2002). Hence, there is an urgent need to make an attempt on the inoculation of halophilic *Azospirillum* species for artificial regeneration of mangrove seedlings (Bashan and Holguin, 1997; Bashan *et al*., 1998). To overcome the poor seedling establishment, the present study aimed to improve the growth of mangrove seedlings (*Ceriops decandra, Avicennia officinalis* and *Rhizophora apiculata*) by using the *Azospirillum* derived from mangrove habitats.

Materials and Methods

Isolation of Azospirillum and chemical constituents: Rhizosphere soil sample from *Rhizophora mucronata* were collected from Manakkudy mangrove region (77° 7'-77° 35'E and 8° 5' -8° 35' N), Tamilnadu, India. The collected samples were transferred to clean and previously unused polythene bags and brought to the laboratory. About 1g of rhizosphere soil was subjected for serial dilution and inoculated into the nitrogen free basal agar medium (Dobereiner *et al*., 1976). The inoculated samples were incubated in an inverted position for 9 days at 30°C. After attaining the visible growth, *Azospirillum* were isolated by repeated streaking in nitrogen free agar plates. The isolates were identified at species level (Holt *et al*., 1994). All the determinants were carried out in triplicate.

***In-vitro* nitrogen fixation and Indole acetic acid (IAA) production by *Azospirillum* :** The pH regimes of 3, 4, 5, 6, 7 and 8 and salinity levels (0, 10, 15, 20, 25, 30 and 35 g l^{-1} NaCl) were prepared in 50 ml nitrogen free broth in different flasks. To calculate the content of IAA the nitrogen free broth was prepared with the addition of 1 g l^{-1} L- tryptophan and 100 mg of yeast extract. 5 ml of *Azospirillum lipoferum, Azospirillum brasilense, Azospirillum halopraeferens, Azospirillum amazonense* and *Azospirillum irakense* cell suspensions (10^8 cells ml^{-1}) were added into each flask and kept for incubation. After 5 days of incubation, the production of IAA was measured (Gorden and Paleg, 1957). In brief, about 25 ml of individual broth was transferred to 500 ml Erlenmeyer flask containing 100 ml of phosphate buffer at pH 7.0 with 0.05 M DL-tryptophan and 5.0 g of sucrose and incubated in dark at 28 ± 1^{0}C for 24 hr filtered through Whatman No.42 filter paper and 50 ml of filtrate were adjusted to pH 3.0 with 2 N HCl. The filtrate was extracted thrice with equal volumes of peroxide free ether at 2^{0}C. The ether was evaporated in vacuum flask evaporator and the residue was dissolved in 2 ml methanol. IAA in the methanol fraction was determined by employing salper reagent. To 1.5 ml of distilled water in a test tube, 0.5 ml of methanol residue was mixed, with 4 ml of fresh salper reagent were rapidly added, kept in complete darkness for 1 hr and read the optical density at 535 nm in a spectrophotometer (220S Hitachi, Japan). To calculate the rate of nitrogen fixation, 25 ml of medium from each salinity level were transferred into 65 ml of serum bottles, and autoclaved at 15 lbs for 15 minutes. Liquid cell suspension (10^8 cells.ml^{-1}) of each *Azospirillum* sp were inoculated into the medium. After that, the bottles were sealed with rubber stopper. From these bottles, 10% of the gaseous phase was removed and the same volume of acetylene gas was injected. After 12 hrs of incubation, the amount of ethylene was analyzed in the gas chromatograph (Agilent 7000 QP2010S, Schimadzu, Japan). Control bottles contained nutrient medium only. Triplicate samples were maintained for each treatment for each species. Nitrogenase activity is expressed in nanomoles of ethylene, 10^8 $cells^{-1}$ ml^{-1} hr^{-1}. The rate of nitrogen fixation was calculated as per procedure of Hardy *et al.* (1975).

Mangrove seedling treatments :The bacterial species were inoculated into 150 ml of liquid Nfb medium and were cultured at 28 ± 2^{0}C for 9 days in a thermostat shaker. The liquid culture was centrifuged at 12,000 rpm for 15 min in a centrifuge (Hewlett Packard, USA). The pellets were suspended in phosphate buffer and washed repeatedly with the buffer and were resuspended in the same buffer solution. 100ml of suspended culture (10^8 cells ml^{-1}) of *Azospirillum lipoferum, Azospirillum brasilense, Azospirillum halopraeferens, Azospirillum amazonense* and *Azospirillum irakense* were mixed separately with 1kg of sterilized soil and were kept in sterilized polythene bags. Propagules of mangrove plants [*Avicennia officinalis* L., *Rhizophora apiculata* Blume (20 ± 2 cm length), *Ceriops decandra* (Griff.) Din Hou. (8 ± 2 cm length)] were sown. Irrigation was done regularly with 15ppt of sterile saline water for mangrove seedlings. The soil without the bacterial inoculums was kept as a control. After 45 days of sowing, the root and shoot growth parameters *viz*., number of roots, maximum root length, average root length, average root biomass, maximum shoot length, average shoot length, average shoot biomass, leaf area and the level of pigments *viz*. Chl *a*, Chl *b*, carotenoids, total chl and biochemical constituents *viz*., protein (Lowry *et al*., 1951), sugars (Dubios *et al*., 1956) and amino acids (Moore and Stein, 1948) were analyzed. The results were statistically analyzed by following two-way analysis of the variance (ANOVA) for significance ($p<0.05$) analysis.

Results and Discussion

Five different species of *Azospirillum* were identified from the rhizosphere soil sample of *Rhizophora mucronata.* Among them *Azospirillum lipoferum* (60%) was dominant followed by *Azospirillum brasilense* (25%), *Azospirillum irakense* (5%), *Azospirillum halopraeferens* (5%) and *Azospirillum amazonense* (5%) (Table 1). All the five *Azospirillum* species showed maximum production of IAA and the rate of nitrogen fixation at pH 7.0. However, *A. brasilense* showed maximum IAA production (19.8mg.ml^{-1}) and nitrogen fixation (5.9 $C_2H_2hr^{-1}$) at pH 7.0. Moreover, the five *Azospirillum* species showed maximum production of IAA and the rate of nitrogen fixation at the salinity level of 10 g l^{-1} NaCl. But, *A. brasilense* showed maximum IAA production (18mg ml^{-1}) and nitrogen fixation (5.7 C_2H_2 hr^{-1}) at the salinity 15 g l^{-1} (Fig. 1 and 2).

The effect of *Azospirillum* species on *Avicennia officinals* seedlings (Table 2 and 3) revealed that the average number of primary roots (6.25%) and protein (17.93%) were significantly enhanced by the inoculation of *A. lipoferum* than the other

Table- 1: Species level identification of *Azospirillum* bacterial isolates from Rhizosphere soil

Characteristics	*A. lipoferum*	*A. brasilense*	*A. halopraeferens*	*A. amazonense*	*A. irakense*
Dominance (%)	60	25	5	5	5
Pleomorphic cells in alkaline N-free semisolid medium	+	-	-	-	-
Lateral flagella formed in addition to polar flagella when grown on agar medium	+	+	-	-	+
Growth at optimum temperature (^{0}C)	37	37	41	35	30 – 33
Growth at pH 6.0	+	+	+	+	+
Growth in presence of 3% NaCl	+	+	+	+	+
Biotin requirement	+	-	+	-	-
Growth on Potato (BMS) agar. Colony type on potato agar					
White, flat with raised margin	-	-	-	+	-
Pink, raised, curled	+	+	-	-	-
Brownish, small, raised, smooth	-	-	-	-	-
Sole carbon sources for growth in N-deficient semisolid medium					
Glucose	+	-	-	+	
Sucrose	-	-	-	+	
a-Ketoglutarate	-	+	+	-	
Citrate	+	+		-	
Carbon source utilization (API gallery method)					
Glucose	+	+	-	+	+
Sucrose	-	-	-	+	+
Myo-inositol	-	-	-	+	+
Dissimilation of NO_3^- to NO_2^-	+	+	+	-	+
NO_2^- to N_2O	+	-		-	-
Pectin hydrolysed in seven days	-	-	-	-	+

+: growth, -: no growth

inoculations. The maximum root length (29.55%), average root length (7.39%), total Chl (55.36%), carotenoids (28.57%), Chl *b* (37.50%), carbohydrates (90.91%) and total amino acids (78.95%) were significantly ($p<0.05$) enhanced with the inoculation of *A. brasilense.* Inoculation of *A. halopraeferens* significantly ($p<0.05$) enhanced the level of average root biomass (40.74%). The average number of primary roots (6.25%) and maximum shoot length (20.25%) were significantly ($p<0.05$) enhanced by the inoculation of *A. amazonense.* Inoculation of *A. irakense* significantly ($p<0.05$) enhanced the average shoot length (22.74%), average shoot biomass (20.19%) and average leaf area (38.72%) in *A. officinalis* seedlings.

The effect of *Azospirillum* species on the growth parameters of *Ceriops decandra* (Table 4 and 5) seedlings revealed that the level of maximum root length (38.03%), average root length (42.33%), maximum shoot length (45.59%), average shoot length (40.15%), average leaf area (45.19%), total Chl (20%) and carbohydrate (90.72%) were significantly ($p<0.05$) enhanced by the inoculation of *A. lipoferum.* Inoculation of *A. brasilense* significantly increased the of average number of primary roots (40%), average biomass (44.44%), average shoot biomass (55.56%), total Chl (20%), Chl *b* (77.78%) and carotenoid (1.54%) levels. The level of total Chl (20%) was significantly ($p<0.05$) enhanced by the inoculation of *A. halopraeferans.* Inoculation of *A. amazonense* enhanced the growth parameters of carotenoid (1.54%), protein (34.48%) and total amino acids (33.33%). The Chl *a* (50%) and total amino acids (33.33%) levels were significantly ($p<0.05$) increased by the inoculation of *A. irakense.*

The effect of *Azospirillum* species on the growth parameters of *R. apiculata* showed that, the average root length (20.99%), maximum shoot length (17.57%), Chl *a* (60%) and protein (50%) were significantly ($p<0.05$) enhanced by the inoculation of *A. lipoferum.* The maximum root length (27.66%) and Chl *a* (60%) were significantly enhanced by the inoculation of *A. brasilense.* Inoculation of *A. halopraeferens* significantly ($p<0.05$) increased the Chl *a* (60%) content. The average shoot length (13.57%) and average shoot bio mass (14.84%) were significantly enhanced by the inoculation of *A. amazonense.* The average number of primary roots (23.08%), average root biomass (15.52%), average shoot biomass (15.30%), carbohydrate (20%) and total amino acids (44.44%) were significantly ($p<0.05$) enhanced by the inoculation of *A. irakense* (Table 6 and 7).

Among five *Azospirillum* sp., *Azospirillum brasilense* was the most dominant group whereas *A. lipoferum* was the most dominant one

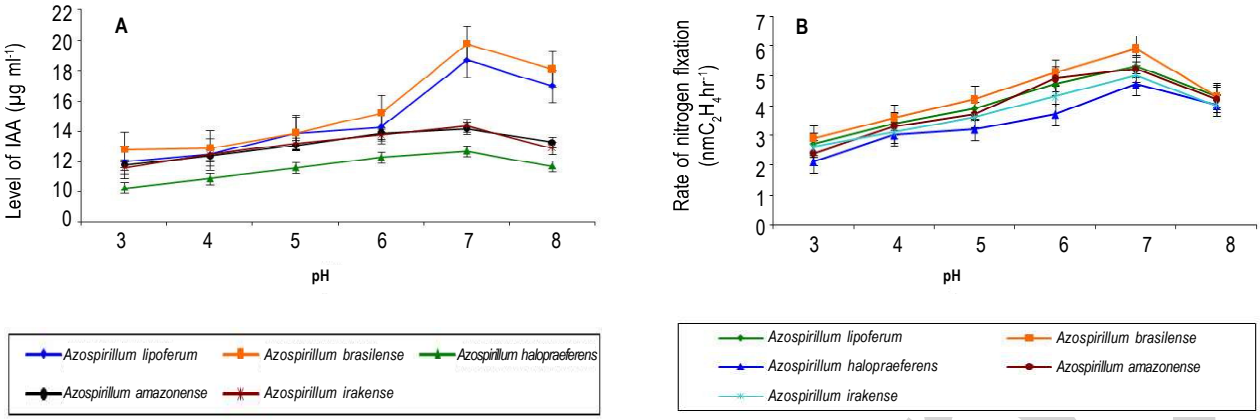

Fig. 1: Effect of different pH on the (A) Indole acetic acid (IAA) production and (B) Nitrogen fixation by the *Azospirillum* species

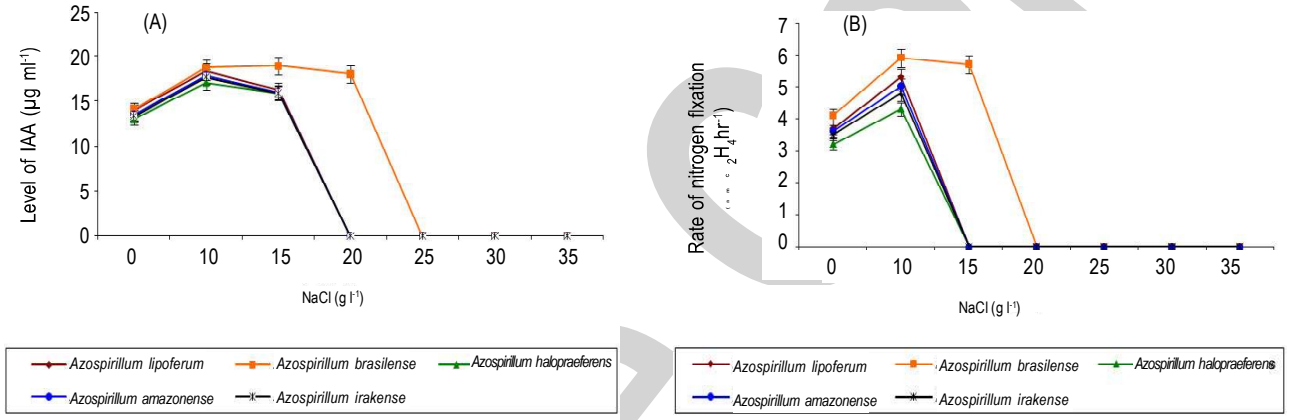

Fig. 2: Effect of different salinity regimes on the (A) Indole acetic acid (IAA) production and (B) Nitrogen fixation by the *Azospirillum* species

Table- 2: Effect of five *Azospirillum* species on the root and shoot growth parameters of *Avicennia officinalis* seedlings

Name of the bacterial species	Average number of primary root	Maximum root length (cm)	Average root length (cm)	Average root biomass (g)	Maximum shoot length (cm)	Average shoot length (cm)	Average shoot biomass (g)
Azospirillum	16.00±2.34*	10.40±1.98^{ns}	6.48±0.97*	0.16±0.02*	41.20±7.65*	38.60±6.53*	0.97±0.04*
lipoferum	(6.25)	(-19.23)	(3.24)	(0.00)	(8.25)	(16.58)	(14.43)
Azospirillum	13.00±1.63	17.60±3.87*	6.77±2.98*	0.18±0.02*	42.90±5.01*	34.54±2.65*	1.03±0.18*
brasilense	(-15.38)	(29.55)	(7.39)	(11.11)	(11.89)	(6.77)	(19.42)
Azospirillum	12.00±2.54	12.80±2.98*	5.13±0.94	0.27±0.04*	42.80±8.92*	35.12±7.98*	0.85±0.01*
halopraeferans	(-25.00)	(3.13)	(-22.22)	(40.74)	(11.68)	(8.31)	(2.35)
Azospirillum	16.00±4.91*	14.30±7.93*	6.42±1.95*	0.25±0.03*	47.40±8.93*	38.76±7.82*	1.03±0.03*
amazonense	(6.25)	(13.29)	(2.34)	(36.00)	(20.25)	(16.92)	(19.42)
Azospirillum	15.00±1.96*	12.60±2.98*	6.41±1.97*	0.23±0.01*	46.40±9.04*	41.68±9.57*	1.04±0.91*
irakense	(0.00)	(1.59)	(2.18)	(30.43)	(18.53)	(22.74)	(20.19)
Control	15.00±1.46	12.40±1.98	6.27±0.92	0.16±0.03	37.80±8.98	32.20±7.82	0.83±0.02

Values are mean of three samples ±SM. Values in the parenthesis are percentage increase or decrease over control; *(p<0.05)* significant from control group. ns: Non significant.

Table- 3: Effect of five *Azospirillum* species on biochemical parameters of *Avicennia officinalis* seedlings

Name of the bacterial species	Average leaf area (cm^2)	Total Chl ($g\ g^{-1}$)	Chl *a* ($g\ g^{-1}$)	Chl *b* ($g\ g^{-1}$)	Carotenoid ($g\ g^{-1}$)	Carbohydrate ($g\ g^{-1}$)	Protein ($g\ g^{-1}$)	Total amino acids ($g\ g^{-1}$)
Azospirillum lipoferum	8.13±2.91* (5.78)	0.03±0.001* (21.88)	0.00±0.00^{ns} (-400.00)	0.009±0.001* (44.44)	0.68±0.02* (11.76)	0.20±0.02* (90.00)	0.02±0.001* (17.39)	0.02±0.001* (77.78)
Azospirillum brasilense	9.75±2.17* (21.44)	0.06±0.001* (55.36)	0.008±0.001^{ns} (-150.00)	0.008±0.001* (37.50)	0.84±0.05* (28.57)	0.22±0.09* (90.91)	0.02±0.001* (5.00)	0.02±0.001* (78.95)
Azospirillum halopraeferans	8.83±2.09* (13.25)	0.02±0.001^{ns} (-19.05)	0.02±0.001^{ns} (-17.64)	0.003±0.001^{ns} (-66.67)	0.52±0.12^{ns} (-15.38)	0.09±0.01* (77.78)	0.02±0.001* (9.52)	0.009±0.001* (55.56)
Azospirillum amazonense	10.70±2.09* (28.41)	0.02±0.001^{ns} (-8.70)	0.02±0.001^{ns} (-11.11)	0.005±0.001* (0.00)	0.73±0.10* (17.81)	0.02±0.001* (0.00)	0.02±0.001* (0.00)	0.007±0.001* (42.86)
Azospirillum irakense	12.50±1.95* (38.72)	0.03±0.01* (3.85)	0.02±0.001* (0.00)	0.005±0.001* (0.00)	0.58±0.02^{ns} (-3.45)	0.04±0.001* (50.00)	0.02±0.001* (0.00)	0.005±0.001* (20.00)
Control	7.66±2.91	0.03±0.001	0.02±0.001	0.005±0.001	0.60±0.05	0.02±0.001	0.02±0.001	0.004±0.001

Data represents the mean ±SM of three samples. Values in the parenthesis are percentage increase or decrease over control; *(p<0.05)* significant from control group. ns: Non significant.

Table- 4: Effect of five *Azospirillum* species on the root and shoot growth parameters in *Ceriops decandra* seedlings

Name of the bacterial species	Average number of primary root	Maximum root length (cm)	Average root length (cm)	Average root biomass (g)	Maximum shoot length (cm)	Average shoot length (cm)	Average shoot biomass (g)
Azospirillum lipoferum	3.00±0.95* (0.00)	14.20±2.87* (38.03)	10.23±4.92* (42.33)	0.06±0.01* (16.67)	6.80±2.01* (45.59)	5.28±2.91* (40.15)	0.18±0.03* (11.11)
Azospirillum brasilense	5.00±0.92* (40.00)	9.70±1.78* (9.28)	5.63±0.92^{ns} (-4.80)	0.09±0.01* (44.44)	5.40±0.92* (31.48)	3.84±0.92* (17.71)	0.36±0.01* (55.56)
Azospirillum halopraeferans	4.00±0.72* (25.00)	11.60±2.83* (24.14)	6.55±0.97* (9.92)	0.07±0.01* (28.57)	3.70±0.93* (0.00)	2.74±0.91^{ns} (-15.33)	0.15±0.02^{ns} (-6.67)
Azospirillum amazonense	4.00±0.32* (25.00)	8.40±0.72* (-4.76)	6.11±1.92* (3.44)	0.06±0.01* (16.67)	3.60±0.91^{ns} (-2.78)	2.16±0.96^{ns} (-46.30)	0.18±0.01* (11.11)
Azospirillum irakense	4.00±0.85* (25.00)	11.80±0.49* (25.42)	3.98±0.74^{ns} (-48.24)	0.05±0.01* (0.00)	4.90±0.93* (24.49)	3.64±0.93* (13.19)	0.20±0.01* (20.00)
Control	3.00±0.94	8.80±1.91	5.90±0.86	0.05±0.01	3.70±0.92	3.16±0.47	0.16±0.01

Data represents the mean ±SM of three samples. Values in the parenthesis are percentage increase or decrease over control; *(p<0.05)* significant from control group. ns: Non significant.

Table- 5: Effect of five *Azospirillum* species on biochemical changes in *Ceriops decandra* seedlings

Name of the bacterial species	Average leaf area (cm^2)	Total Chl ($g\ g^{-1}$)	Chl *a* ($g\ g^{-1}$)	Chl *b* ($g\ g^{-1}$)	Carotenoid ($g\ g^{-1}$)	Carbohydrate ($g\ g^{-1}$)	Protein ($g\ g^{-1}$)	Total amino acids ($g\ g^{-1}$)
Azospirillum lipoferum	13.50±1.38* (45.19)	0.005±0.001* (20.00)	0.004±0.001* (25.00)	0.00±0.00^{ns} (-100.00)	0.06±0.01^{ns} (-128.57)	0.10±0.01* (90.72)	0.01±0.001^{ns} (-72.73)	0.002±0.001* (0.00)
Azospirillum brasilense	10.10±2.20* (26.73)	0.005±0.01* (20.00)	0.004±0.01* (25.00)	0.009±0.01* (77.78)	0.13±0.02* (1.54)	0.09±0.01* (89.89)	0.02±0.01^{ns} (-26.67)	0.001±0.001^{ns} (-100.00)
Azospirillum halopraeferans	8.20±2.94* (9.76)	0.005±0.01* (20.00)	0.004±0.001* (25.00)	0.001^{ns} (-100.00)	0.13±0.02^{ns} (-2.40)	0.08±0.03* (88.31)	0.007±0.001^{ns} (-171.43)	0.002±0.001* (0.00)
Azospirillum amazonense	8.30±1.93* (10.84)	0.004±0.001* (0.00)	0.004±0.001* (25.00)	0.001±0.001^{ns} (-100.00)	0.13±0.04* (1.54)	0.15±0.06* (94.00)	0.03±0.01* (34.48)	0.003±0.001* (33.33)
Azospirillum irakense	8.90±1.93* (16.85)	0.003±0.001^{ns} (-33.33)	0.002±0.001* (50.00)	0.005±0.001* (60.00)	0.08±0.01^{ns} (-58.02)	0.09±0.01* (90.32)	0.02±0.01* (17.39)	0.003±0.001* (33.33)
Control	7.40±1.92	0.004±0.001	0.003±0.001	0.002±0.001	0.13±0.06	0.009±0.001	0.02±0.01	0.002±0.001

Data represents the mean ±SM of three samples. Values in the parenthesis are percentage increase or decrease over control; *(p<0.05)* significant from control group. ns: Non significant.

Table- 6: Effect of five *Azospirillum* species on the root and shoot growth parameters of *Rhizophora apiculata* seedlings

Name of the bacterial species	Average number of primary root	Maximum root length (cm)	Average root length (cm)	Average root biomass (g)	Maximum shoot length (cm)	Average shoot length (cm)	Average shoot biomass (g)
Azospirillum lipoferum	11.00±2.78* (9.09)	12.90±2.65^{ns} (-5.43)	8.10±3.87* (20.99)	0.39±0.06^{ns} (-25.64)	29.60±7.92* (17.57)	25.40±4.91* (12.20)	1.53±0.48^{ns} (-1.31)
Azospirillum brasilense	11.00±3.98* (9.09)	18.80±4.91* (27.66)	5.20±0.82^{ns} (-23.08)	0.42±0.06^{ns} (-16.67)	26.40±3.92* (7.58)	22.60±3.01* (1.33)	1.49±0.07^{ns} (-4.03)
Azospirillum halopraeferans	10.00±0.96* (0.00)	15.90±1.02* (14.47)	7.00±0.91* (8.57)	0.22±0.04^{ns} (-122.73)	26.90±5.92* (9.29)	23.20±4.92* (3.88)	1.45±0.04^{ns} (-6.90)
Azospirillum amazonense	10.00±0.62* (0.00)	12.50±2,89* (-8.80)	6.90±0.92* (7.25)	0.48±0.57^{ns} (-2.08)	28.90±7.91* (15.57)	25.80±4.81* (13.57)	1.82±0.49* (14.84)
Azospirillum irakense	13.00±2.93* (23.08)	13.70±2.93* (0.73)	7.00±0.92* (8.57)	0.58±0.03* (15.52)	26.90±5.93* (9.29)	25.70±7.82* (13.23)	1.83±0.07* (15.30)
Control	10.00±1.93	13.60±1.03	6.40±0.49	0.49±0.03	24.40±4.82	22.30±0.05	1.55±0.49

Data represents the mean ±SM of three samples. Values in the parenthesis are percentage increase or decrease over control; *(p<0.05)* significant from control group. ns: Non significant.

Table- 7: Effect of five *Azospirillum* species on biochemical parameters of *Rhizophora apiculata* seedlings

Name of the bacterial species	Average leaf area (cm^2)	Total Chl ($g\ g^{-1}$)	Chl *a* ($g\ g^{-1}$)	Chl *b* ($g\ g^{-1}$)	Carotenoid ($g\ g^{-1}$)	Carbohydrate ($g\ g^{-1}$)	Protein ($g\ g^{-1}$)	Total amino acids ($g\ g^{-1}$)
Azospirillum lipoferum	20.50±2.87* (14.63)	0.006±0.001^{ns} (-33.33)	0.005±0.001* (60.00)	0.001±0.001^{ns} (-100.00)	0.16±0.03^{ns} (-93.75)	0.14±0.02* (14.29)	0.04±0.01* (50.00)	0.007±0.001* (28.57)
Azospirillum brasilense	21.00±2.98* (16.67)	0.007±0.001^{ns} (-14.28)	0.005±0.001* (60.00)	0.001±0.001^{ns} (-100.00)	0.21±0.001^{ns} (-47.61)	0.14±0.01* (14.29)	0.02±0.01* (0.00)	0.005±0.001* (0.00)
Azospirillum halopraeferans	17.80±3.92* (1.69)	0.007±0.002^{ns} (-14.28)	0.005±.001* (60.00)	0.001±0.001^{ns} (-100.00)	0.16±0.07^{ns} (-93.75)	0.12±0.06* (0.00)	0.03±0.01* (33.33)	0.004±0.001^{ns} (-25.00)
Azospirillum amazonense	20.10±4.02* (12.94)	0.005±0.001^{ns} (-60.00)	0.004±0.001* (50.00)	0.001±0.001^{ns} (-100.00)	0.14±0.02^{ns} (-121.42)	0.12±0.03* (0.00)	0.02±0.01* (0.00)	0.004±0.001^{ns} (-25.00)
Azospirillum irakense	20.50±5.79* (14.63)	0.005±0.001^{ns} (-60.00)	0.004±0.001* (50.00)	0.001±0.001^{ns} (-100.00)	0.15±0.08^{ns} (-106.66)	0.15±0.07* (20.00)	0.03±0.01* (33.33)	0.009±0.001* (44.44)
Control	17.50±1.39	0.008±0.001	0.002±0.001	0.002±0.001	0.31±0.05	0.12±0.03	0.02±0.01	0.005±0.001

Values are the mean of three replecales ±SM. Values in the parenthesis are percentage increase or decrease over control; *(p<0.05)* significant from control group. ns: Non significant.

in the Pichavaram mangrove ecosystem (Ravikumar *et al*., 2002). Earlier reports revealed that the species diversity varied with the environment; for instance, *A. lipoferum* and *B. megaterium* has been originally isolated from roots of maize and rice plants (El-Komy, 2005). The complete inhibition of IAA production and nitrogen fixing activity was observed at higher salinity levels. Ravikumar *et al.* (2004) reported that the growth of *Azotobacter* species *viz. A. chroococcum, A. vinelandii, A. beijerinckii* were maximum at higher salinity levels but the IAA production and nitrogen fixation was completely arrested at these levels. Growth promoting effect of *Azospirillum* on the growth of mangroves reveals that an enhanced root growth, shoot growth, pigments and organic constituents in mangrove seedlings are due to several reasons. The most important reason was the biological N_2 fixation exerted by the bacteria (Chandrasekar *et al.*, 2005). Watt *et al.* (2006) reported that biological nitrogen fixation enhanced the water and mineral uptake. Production of phytohormone and nitrate reduction in the rhizosphere regions by inoculated *Azospirillum* species (Bashan and Holguin, 1997; Steenhoudt and Van der Leyden, 2000). Upon the *Azospirillum* inoculation, the root displayed a significant increase in the number and length of root hairs, rate of appearance and number of lateral roots, diameter and length of lateral and adventitious roots and root surface area (Mandal and Sinha, 2004). The increased root development leads to an increased root surface that could improve plant nutrition and thus would be a key factor for plant. Production of auxin which may increase the yield, in some instances, production of auxin regulators (sugars) could be influenced by the compounds released by the plant roots (Arshad and Frakenberger, 1998). Bloom *et al.* (2003) reported that molecules such as ammonium, nitrate and NO are potentially involved in root development and proliferation. It has been largely known that *Azospirillum* can produce NO at low O_2 pressure by denitrification

process (Hartmann and Zimmer, 1994). Optimum bacterial concentration (10^8 cells ml^{-1}) of *Azospirillum* also provoked root elongation (Burelle *et al.*, 2006). Ravikumar *et al.* (2002) reported that the halophilic phosphobacteria isolated from the Pichavaram mangrove enhanced the root and shoot growth characteristic, level of pigments (chlorophyll and carotenoids) in the mangroves seedlings. The accumulation of the photosynthetic pigments reveals a rich light harvesting efficiency of the treated plants and hence, an increase in photochemical activity in the treated seedlings may be assumed. The growth promoting effect of *Azospirillum* sp is attributed by the ability to fix atmospheric nitrogen and making it available to the mangrove seedlings. In the present study, *Azospirillum* sp. having the capability of fixing nitrogen and also synthesising the phytohormone (Indole acetic acid) in *in vitro* condition are required for better growth and pigment production of mangrove seedlings (Ravikumar *et al.*, 2002). Dey *et al.* (2004) reported that the increase in concentration of IAA directly promoted the chlorophyll content in *Anabaena* species. It is concluded from the present study that inoculation of *Azospirillum* in the soil enhanced the growth performance of mangroves. Based on growth parameters, *Azospirillum brasilense* was found better for the growth of *Avicennia officinalis* and *Ceriops decandra* seedlings, but *Azospirillum irakense* was found better for *Rhizophora apiculata* seedlings.

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