BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, October 2014. Vol. **11**(Spl. Edn. 1), p. 179-185

Production of Indole Acetic Acid and Plant Growth Promotion by Rhizobacteria from a Less Studied Marine Ecosystem

M. Jayaprakashvel[1,3], V. Kiran Kumar[2], Jainul Abideen[2], Swarnakala[1], M. Venkatramani[1] and A. Jaffar Hussain[1,3]

[1]Department of Marine Biotechnology, AMET University (u/s 3 of UGC Act 1956)
135, East Coast Road, Kanathur, Chennai-603112, India
[2] Department of Environmental Sciences, Sri Venkateswara University
Tirupati, Andhra Pradesh-517 502, India.
[3]Centre for Marine Bioprospecting, AMET University (u/s 3 of UGC Act 1956)
135, East Coast Road, Kanathur, Chennai-603112, India

doi: http://dx.doi.org/10.13005/bbra/1408

(Received: 15 August 2014; accepted: 10 October 2014)

Coastal sand dunes are one of the less explored marine ecosystems. The environmental conditions prevailing in these ecosystems make the microorganisms and other living forms more competent and may have good potential. So, bioprospecting of coastal sand dune microorganisms is a worthy one. In this paper, we report the ability of coastal sand dune rhizobacteria to produce indole acetic acid and their potential to increase the seedling growth of crop plants. A total of 47 rhizobacterial strains were isolated from 5 rhizosphere samples collected from the coastal sand dunes in Uthandi Beach, Chennai. Among the 47, 22 rhizobacteria were found to be fluorescent pseudomonads. Among 47, 7 were found to be gram positive and remaining were gram negative bacteria. 11 among these 47 rhizobacteria were found to produce plant growth hormone indole acetic acid (IAA) extra-cellularly. Quantitative tests resulted in short listing strains AMET9318, AMET9323 and AMET9345 as highest IAA producers. All these three IAA producing strains were demonstrated for their remarkable ability to increase seedling length and seedling weight of wheat, green gram, mustard, black gram and kidney bean through seedling growth chamber studies. Thus, the present study concludes the plant growth promotion potentials of coastal sand dune rhizobacteria which can be used as biofertilizers in coastal saline soils.

Key words: coastal sand dunes, rhizobacteria, indole acetic acid, plant growth promotion, Biofertilizers

Coastal sand dunes (CSD) are one of the least explored marine ecosystems for beneficial microorganisms. They are distributed throughout the world except in Antarctica; they serve as an ecological niche between marine and terrestrial systems (Padmavathy and Anbarashan, 2011). They comprise of a range of habitats which are of crucial ecological and economic importance (Martinez *et al* 1997; Rodrigues *et al*., 2011). Plants that grow on beaches and sand dunes form communities known as coastal dune vegetation. Growth, survival and heterogeneity of CSD vegetation spatially and temporally influenced by environmental factors such as temperature, desiccation, low moisture retention, sand erosion, sand accretion, salinity and salt spray (Watkinson and Davy 1985, Maun 1994; Shridhar and Bhagya, 2007). CSD flora has a wide range of applications in nutrition, medicine, industry and agriculture (Shridhar and Bhagya, 2007).

* To whom all correspondence should be addressed.
E-mail: jayaprakashvel@gmail.com

Coastal sand dune vegetation is rarely explored for microbiological studies. The physiochemical, environmental and nutritional stresses in the CSD and its associated vegetation may pose severe pressure on the survival and sustenance of microflora associated with CSD vegetation. Though not adequate, the microorganisms from this ecosystem are explored for many beneficial bioactivities. Endophytic bacteria associated with the roots of coastal sand dune plants were isolated, taxonomically characterized, and tested for their plant growth-promoting activities like antifungal activity against phytopathogens and production of extracellular enzymes (Shin *et al*., 2007). Godinho *et al*. (2007) have isolated and characterized bacteria from coastal sand dunes with plant growth promotion potentials on egg plant. Hydrogen cyanide mediated biocontrol potential of *Pseudomonas* sp. AMET1055 isolated from the rhizosphere of coastal sand dune vegetation was also demonstrated (Jayaprakashvel *et al*., 2010) Siderophore producing bacteria were isolated from a sand dune ecosystem and the effect of sodium benzoate on siderophore production by a potential isolate was characterized (Gaonkar *et al*., 2012). Thus, few researchers have indicated the plant growth promotion potentials of CSD vegetation associated bacteria. However, a deep insight in to the mechanism of plant growth promotion and demonstration of the same in crop plants is not yet attempted comprehensively. In the present study, we have identified three plant growth promoting *Pseuomonas* spp. with the ability to produce IAA and their plant growth promotion potential on five crop plant seedlings through a seedling growth chamber study.

MATERIALS AND METHODS

Isolation of rhizobacteria from coastal sand dunes

Ten gram of rhizosphere soil along with root bits of coastal sand dune plants were added to 95 ml of sterile distilled water in a conical flask. This suspension was serially diluted and spread plated on King's B agar (KBA) medium (King *et al*., 1954). The plates were incubated at room temperature (30 ± 2ºC) for 48 h. Single colonies of different morphology were picked and subcultured again on KBA medium and observed for the production of fluorescent pigments under UV transillumination. The colonies that fluoresced under UV light were identified as fluorescent pseudomonads (Jayaprakashvel *et al.*, 2010). Standard gram staining methodology as detailed by Cuappuccino and Sherman (2004) was followed to characterize the rhizobacteria for their cell wall characteristics.

Screening of Indole Acetic Acid (IAA)

All the coastal sand dune rhizobacteria were grown in 5 mL nutrient broth medium amended with 0.1 % tryptone in test tubes for 24 h. After incubation period, bacterial cultures were harvested and centrifuged at 10000 RPM for 15 min at 4°C. Two drops of *o*- phosphoric acid were added to 2 mL of cell free supernatant and the development of color was observed. The presence of a pink color showed the production of IAA (Gupta *et al.*, 2002). The cell free supernatant was also used for quantification of IAA.

Quantification of IAA production

One ml of supernatant was mixed with 1 ml of Salkowski's reagent (12 g $FeCl_3$ in 429 ml H_2SO_4 and make up to 1 liter with distilled water). After 20 minutes incubation at room temperature in complete darkness, the absorbance at 535 nm was determined. IAA concentrations were determined using standard graphs for authentic IAA (Sigma) (Gutierrez *et al*., 2009).

Identification of IAA producing CSD rhizobacteria by biochemical tests

The three best IAA producing rhizobacteria from coastal sand dune vegetation viz., AMET 9318, AMET 9323 and AMET 9345 were subjected to various biochemical and staining techniques such as gram staining, motility, MRVP, citrate utilization, gelatin hydrolysis etc by following standard methodologies described by Cappuccino and Sherman (2004) and the results were interpreted with the key provided in the Bergy's Manual of Determinative Bacteriology (Holt *et al.*, 2004).

Effect of selected IAA producing CSD rhizobacteria on seedling growth

Three selected IAA producing CSD rhizobacteria viz., *Pseudomonas* sp. AMET 9318, Pseudomonas sp. AMET 9323 and *Pseudomonas* sp. AMET 9345 were grown in 100 ml of nutrient broth medium amended with 0.1 % tryptone in 500 ml conical flasks for 24 h as static cultures. The

broth culture as such was used as inoculums for the seedling growth studies. Seeds of crops plants such as Wheat, Green gram, Mustard, Black gram and Kidney bean were used. Before bacterial treatments, the seeds were surface sterilized by soaking in 0.5 % sodium hypochlorite solution for 5 minutes followed by washing twice in sterile distilled water and air died on blotting papers. Plastic cups with 125 g of sterile soil/cup were used in this experiment. Seed bacterization treatment was given to the respective seeds by immersing the 10 of seeds in 25 ml of broth cultures of selected bacteria containing 0.3% of glycerol for adhesision. After 4 h, the seeds were transferred to experimental plastic cups filled with sterile soil. After 3 days, 5 ml broth cultures of selected bacteria were drenched in plastic cups with soil and bacterized seeds for soil drenching treatment. Seedlings were sprayed with water on alternate days and no other organic or inorganic fertilizers used. Experiment was conducted with three replicates. The seedling growth parameters such as germination percentage, seedling length and seedling fresh weights were recorded (Jayaprakashvel, 2008). Vigour index was calculated for each treatment using the formula of Abdul-Baki and Anderson (1973).

Vigour Index = (shoot length + root length) x germination percentage.

RESULTS AND DISCUSSION

Saline soils are one of the major issues for agricultural productivity in this population exploding era. Increase in soil salinity turns agronomically useful lands into unproductive areas. The accumulation of soluble salts in the soil can inhibit plant growth by increasing the osmotic potential of interstitial water, inducing ion toxicity and nutrient imbalances in plants. Moreover, increased salinity results in reduced plant photosynthesis, protein synthesis and lipid metabolism and thus causing huge yield loss of many crops. The United Nations Environment Program estimates that approximately 20 % of agricultural land and 50 % of cropland in the world is salt-stressed. Soil salinisation is reducing the area that can be used for agriculture by 1–2 % every year (Wu, 2009; Paul and Lade, 2014). Several microbes promote plant growth, and many microbial products that stimulate plant growth have been marketed. In this review we restrict ourselves to bacteria that are derived from and exert this effect on the root. Such bacteria are generally designated as PGPR (plant-growth-promoting rhizobacteria) (Lugtenberg and Kamilova, 2009). Generally, PGPR facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Ahemad and Kibret, 2014).

Coastal sand dunes are one of the most common ecosystems in the Marine Environment of different parts of the world. They constitute a variety of microenvironments due to substrate mobility and physical processes. Plants establishing on coastal sand dunes are subjected to several environmental stresses such as high solar radiation, nutrient deficiency, drought, salt spray and high winds which affect their growth, survival and community structure (Oosting and Billings,1942; Arun *et al.*, 1999). However, exploration of coastal sand dune associated bacteria for beneficial activities are very scarce despite of their potential (Jayaprakashvel *et al.*, 2010). Hence, these ecosystems are often referred

Table 1. Isolation of marine bacteria from the rhizosphere of coastal sand dune plants

S. No.	Location	Sample	Bacterial Population (log cfu)	No. of strains isolated
1	Uthandi-Site 1	*Ipomoea* Rhizosphere	7.09	12
2	Uthandi-Site 1	*Spinifex* Rhizosphere	6.4	7
3	Uthandi Site 2	*Canavalia* Rhizosphere	7.03	12
4	Uthandi Site 3	*Ipomoea* Rhizosphere	6.54	5
5	Uthandi Site 3	*Spinifex* Rhizosphere	6.95	11
Total				47

as neglected marine ecosystem (Arun *et al*., 1999). We have been exploring the microbial diversity and bioprospecting of coastal sand dunes for the past few years (Jayaprakashvel *et al*., 2010 a&b; Primiya *et al*., 2011; Sangeetha *et al*., 2012). In this context, the present work was undertaken to explore the plant growth promotion potential of rhziobacteria from the coastal sand dune plants.

In this context, the present work was undertaken to isolate rhizobacteria from the rhizosphere of coastal sand dune plants. In and around the study area, the coastal sand dune plants such as *Ipomoea* sp., *Canavalia* sp., and *Spinifex* sp. were dominant flora (Jayaprakashvel, *et al*., 2010; Sangeetha *et al*., 2011). A total of 5 rhizosphere soil samples were collected from three different locations along the coastal area near Uthandi Chennai in the Bay of Bengal. The bacterial populations were in the range of 6.4-7.09 log cfu. Comparatively the rhizosphere of *Ipomoea* sp. and *Canavalia* sp. had more number of colonies with distinct morphologies and hence more strains were isolated from these samples. Altogether, 47 rhizobacteria were isolated from these five rhizosphere soil samples (Table 1).). They were also characterized for Gram's reaction. Among 47, 7 were found to be gram positive and remaining were gram negative bacteria. Similarly, 212 bacteria were isolated from the root and rhizosphere samples of the two plants In this study, the bacterial populations associated with two major sand dune plant species, *Calystegia soldanella* (beach morning glory) and *Elymus mollis* (wild rye), growing along the coastal areas in Tae-An, Chungnam Province, Korea (Park *et al*., 2005).

Fluorescent pseudomonads (FPs), the most intensively studied group among Pseudomonas is one of the most important soil microbial communities having exceptional properties for the enhancement of plant growth (Jayaprakashvel *et al*., 2006). In the present study, all the 47 rhizobacteia were tested for their effect on producing fluorescent pigments in King's B Agar Medim. It has been found that 22 rhizobacteria were found to be fluorescent pseudomonads. Previously, our research group has isolated a total of 24 fluorescent pseuodmonads as pure cultures from eight rhizosphere soil samples collected from Uthandi Beach along the Bay of Bengal, Chennai, India (Sangeetha *et al*., 2011).

Phytohormones are the substances that stimulate plant growth which include indole-3-acetic (IAA) acid (auxin), cytokinins, gibberellins and abscisic acid. PGP inhabit the increase the plant growth through soil nutrient enrichment by nitrogen fixation, phosphate solubilization, siderophore production and phytohormones production. Indole-3-acetic acid (IAA)—IAA is the foremost phytohormone that accelerates plant growth and development by improving root/shoot growth and seedling vigor (Gopalakrishnan *et al*., 2014). In the present study, all the 47 rhizobacteria including 22 fluorescent pseudomonads were subjected to screen for the production of IAA in a semi quantitative assay. It has been found that 11 among 47 strains have produced IAA in their culture filtrates. Among these 11 IAA positive rhizobacteria, three strains namely AMET9318, AMET9323 and AMET9345 have found with potentials to produce more IAA (Table 2). In a previous study, PGPR were isolated from 18 different rhizosphere soil samples of coastal sand dune plants, belonging to the genus *Ipomoea* sp. collected from the Chennai coastal area. From that, 46 morphologically different bacterial strains were isolated. Among 46, 18 strains exhibited the production of Indole Acetic Acid. (IAA) (Muthezhilan *et al*., 2012).

The present study was aimed to study the plant growth promotion potential of rhizobacteria from coastal sand dune plants. Hence, all these three IAA producing strains (AMET9318, AMET9323 and AMET9345) were tested for their effect of seedling growth characteristics in four selected crop plant seedlings such wheat, green

Table 2. Screening of coastal sand dune rhizobacteria for the production of Indole Acetic Acid (IAA)

S. No	IAA positive strain	Absorbance at 530nm
1	AMET9302	0.157
2	AMET9304	0.165
3	AMET9310	0.120
4	AMET9312	0.155
5	AMET9318	0.183
6	AMET9323	0.194
7	AMET9328	0.161
8	AMET9331	0.165
9	AMET9336	0.137
10	AMET9345	0.209
11	AMET9347	0.125

gram, black gram and kidney bean through seedling growth chamber studies. It has been found that both the three strains have remarkably enhanced the germination percentage of all the four crop plant seedlings tested. Similarly, all the strains have enhanced the seedling growth parameters such as shoot and root length and fresh weight (Table 3). In Wheat seedlings, AMET9318 has produced germination highest germination percentage of 85 whem compared to control (50%). One strain AMET9323 reduced the shoot length than the control. AMET9318 has also increased root length (4.6 cm), fresh weight (120 mg) and vigor index (739.5). In Green gram seedlings, also AMET9318 has produced higher seed germination (100%), shoot length (5.8 cm), root length (4.2 cm). The strain AMET9318 also produced highest vigor index of 1000 in green gram seedlings. In black gram all the three strains have enhanced the germination maximum 100% as against the control (80%). Strain AMET9345 has produced highest growth parameters in green gram. In kidney bean, germination percentage was marginally increased from 55% in control in the range up to 80%. Comparatively the strain AMET9323 has produced increased growth parameters in kidney bean.

World over, most of the IAA producing strains were successfully demonstrated for their plant growth promotion. Very recently, bacteria from rhizosphere (*Bacillus pumilus*) and endorhizophere (*Pseudomonas pseudoalcaligenes*) of rice plant capable of producing IAA were found to have enhanced dry weight, plant height, and root length of rice crop in greenhouse (Jha and Subramanian, 2014). Eighty four halotolerant bacterial strains were isolated from the saline habitats and

Table 3. Effect of rhizobacteria treatment on the growth parameters of selected four crop plant seedlings

Treatment	Germination (%)	Shoot Length (in cm)	Root Length (in cm)	Fresh Weight (in mg)	Vigor Index
AMET9318+ Wheat	85	4.1±0.5	4.6±0.4	120±17.3	739.5
AMET9323+ Wheat	75	1.6±0.6	2.7±0.9	80±17.3	322.5
AMET9345+ Wheat	60	4.5±2.3	2.3±0.2	110±22.9	408
Untreated Wheat	50	4.5±1.6	4±1.27	93.3±35	405
AMET9318+ Green gram	100	5.8±2.9	4.2±0.7	233.3±13.2	1000
AMET9323+ Green gram	100	2.8±0.2	3.03±0.4	273.3±25	583
AMET9345+ Green gram	95	4.3±1.1	3.5±1.1	253.3±39.0	741
Untreated Green gram	85	6.03±0.5	3.1±1.7	323.3±13.1	776.05
AMET9318+ Black gram	100	6.7±1.9	3.4±0.3	210±8.6	1010
AMET9323+ Black gram	100	7.1±0.45	2.9±0.2	260±22.9	1000
AMET9345+ Black gram	100	9.5±1.23	3.8±1.1	203.3±21.7	1330
Untreated Black gram	80	3.5±0.61	3.2±1.0	186.6±52.9	536
AMET9318+ Kidney bean	75	3.1±0.5	1.6±0.6	403.3±58.9	352.5
AMET9323+ Kidney bean	75	3.5±0.4	2.4±0.6	453±80	442.5
AMET9345+ Kidney bean	90	3.2±0.6	2.6±1.3	410±34.6	522
Untreated Kidney bean	55	1.6±0.4	1.25±0.1	480±76.9	156.75

characterized for multiple plant growth promoting traits such as indole "3- acetic acid (IAA) production, HCN and siderophore production, ACC deaminase activity and P-solubilization. Five selected halotolerant bacterial strains were demonstrated to ameliorate salt stress in wheat seedlings and enhanced plant growth parameters such as shoot and root length and plant biomass (Ramadoss *et al*., 2013). Both our studies and related studies have concluded that bacteria isolated from saline environments (such as coastal sand dunes) have potential to enhance plant growth under saline stress. Since the selected rhizobacteria in the present study are fluorescent pseudomonads, they can be undoubtedly characterized for agricultural benefits.

CONCLUSION

The present study has demonstrated the plant growth promotion potential of IAA producing rhizobacteria isolated from the rhizosphere of

coastal sand dune plants. This has increased the scope for using these rhizobacteria for the promotion of plant growth in salt influenced soils.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge the support and facilities rendered by the Management of AMET University for the completion of this work. Author MV thank the Management and Authorities of AMET University for Full Time Research Fellowship and encouragement.

REFERENCES

1. Abdul-Baki, A.A., Anderson, J.D., Vigour determination in soybean seed by multiple criteria. *Crop Science.*, 1973; **13:** 630-633.
2. Ahemad, M., Kibret, M., Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University – Science.*, 2014; **26:** 1–20.
3. Arun, A.B., Beena, K.R., Raviraja, N.S., Sridhar, K.R, Coastal sand dunes: a neglected ecosystem. *Current Science.*, 1999; **77:** 19–21.
4. Cappuccino, J.G., Sherman, N., *Microbiology: A laboratory manual. Pearson Education, New Delhi, India*. 2004; 45-200.
5. Gaonkar,T., Nayak, P.K., Garg, S., Bhosle, S., Siderophore-Producing Bacteria from a Sand Dune Ecosystem and the Effect of Sodium Benzoate on Siderophore Production by a Potential Isolate. *The Scientific World Journal.* Article ID 857249, 8. doi:10.1100/2012/857249, 2012.
6. Godinho, A., R. Ramesh, Bhosle, S., Bacteria from sand dunes of Goa promoting growth in Eggplant. *World Journal of Agricultural Sciences.*, 2010; **6:** 555–564.
7. Gopalakrishnan S., Sathya A., Vijayabharathi R., Varshney RK, Laxmipathi Gowda C. L., Krishnamurthy, L. Plant growth promoting rhizobia: *challenges and opportunities. 3 Biotech,* 2014. DOI 10.1007/s13205-014-0241-x.
8. Gutierrez C. K., Matsui G. Y., Lincoln D., Lovell, Cr., Production of the phytohormone indole-3- acetic acid by estuarine species of the Genus Vibrio. *Appl. Environ. Microbiol.* 2009; **75:** 2253–2258.
9. Jayaprakashvel M., Muthezhilan, R., Srinivasan, R., Jaffar Hussain, A., Gopalakrishnan, S., JackyBhagat., Kaarthikeyan, N., Muthulakshmi., Hydrogen cyanide mediated biocontrol potential of *Pseudomonas* sp. AMET1055 isolated from the rhizosphere of coastal sand dune vegetation. *Journal of Advanced Biotechnology* 2010; **9:** 39-42.
10. Jayaprakashvel, M., Muthezhilan, R., Jaffar Hussain, A., Diversity and bioactivities of rhizosphere microflora in the sand dunes of Chennai coast In Abstract of National conference on National Conference on Marine Biodiversity-Present Status and Prospects (MABPSAP 2010), Department of Marine Science, Bharathidasan University held during September 16 – 18, Tiruchirappalli. Tamil Nadu, India, 2010.
11. Jayaprakashvel, M., Ramesh, S., Mathivanan, N., Baby, U.I., Prevalence of fluorescent pseudomonads in the rhizosphere of plantation crops and their antagonistic properties against certain phytopathogens. *J. Plant. Crops* 2006; **34:** 728–732.
12. Jha, Y., Subramanian, RB., Characterization of root-associated bacteria from paddy and its growth-promotion efficacy. *3 Biotech* 2014; **4:** 325-330.
13. Lugtenberg, B., Kamilova, F., Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology.* 2009; **63:** 541-556.
14. Martinez, M.L., Moreno-Casasola, P., Vazquez, G., Effects of disturbance by sand movement and inundation by water on tropical dune vegetation dynamics. *Canadian Journal of Botany*., 1997; **75:** 2005-2014.
15. Maun, M.A., Adaptations enhancing survival and establishment of seedling on coastal dune systems. *Vegetatio*., 1994; **111**; 59-70.
16. Muthezhilan, R., Sindhuja, B.S., Hussain A.J., Jayaprakashvel, M., Efficiency of plant growth promoting rhizobacteria isolated from sand dunes of Chennai coastal area. *Pak J Biol Sci.*, 2012; **15:** 795-799.
17. Oosting, H.J., Billings, W.D., Factors affecting vegetational zonation on coastal dunes. *Ecology*., 1942; **23:** 131-142.
18. Padmavathy, A., Anbarashan, M., Phytomedicinal study of coastal sand dune floras in Puducherry. *Journal of Medicinal Plants Research.* 2011; **5:** 2566-2571.
19. Park, M.S., Jung, S.R., Lee, M.S., Kim, K.O., Do., JO., Lee, K.H., Kim, S.B., Bae, K.S., Isolation and characterization of bacteria associated with two sand dune plant species, *Calystegia soldanella* and *Elymus mollis. The Journal of Microbiology.*, 2005; **43:** 219-227.
20. Paul, D., Lade, H. 2014. Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: *a review. Agronomy for Sustainable*

Development. DOI: 10.1007/s13593-014-0233-6.

21. Primiya, R., Karthigeyan, C.P., Melissa A.C.**,** Jaffar Hussain, A., Jayaprakashvel, M., Bacterial community structure from rhizosphere of coastal sand dune plants and its beneficial bioactivities. In: the National Conference on Marine Biodiversity for New Biotechnological Applications held on December 9, at Hindustan College of Arts and Science, Chennai, 2011.
22. Ramadoss, D., Lakkineni, VK., Bose, P., Ali, S., Annapurna, K., Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. *SpringerPlus January*., 2013; **2:** 6. DOI 10.1186/2193-1801-2-6.
23. Rodrigues, R.S, Mascarenhas, A, Jagtap T.G., An evaluation of flora from coastal sand dunes of India:Rationale for conservation and management, *Ocean and Coastal Management.* 2011; **54:** 181-188.
24. Sangeetha, S.**,** Balram, J., Muthezhilan, R., Jaffar Hussain, A., Jayaprakashvel, M., Influence of salinity on the antifungal activity of marine associated rhizobacteria against *Rhizoctonia solani*. *Journal of Advanced Biotechnology.*, 2011; **11:** 07-11.
25. Sangeetha,S., Moromidevi, Karthik,K., Kaarthikeyan, C., Jaffar Hussain, A., Jayaprakashvel. M., Diversity and beneficial bioactivities of coastal sand dune Actinomycetes. In: National Conference on Marine Environmental Challenges & Coastal Zone Management Strategy held on 13 - 14 February at Bharathidasan University, Tiruchirappalli, 2012.
26. Shin, D.S., Park, M.S., Jung, S., Lee, M.S., Lee, K.H., Bae, K.S., Kim, S.B., Plant growth-promoting potential of endophytic bacteria isolated from roots of coastal sand dune plants. *J Microbiol Biotechnol*., 2007; **17:** 1361-1368.
27. Sridhar, K.R., Bhagya, B., Coastal sand dune vegetation: a potential source of food, fodder and pharmaceuticals. *Livestock Research for Rural Development*., 2007; **19:** Article #84. Retrieved May 18, 2012, from http://www.lrrd.org/lrrd19/6/srid19084.htm.
28. Watkinson, A.R., Davy, A.J., Population biology of salt marsh and sand dune annuals. *Vegetatio.*, 1985; **62:** 487-497.
29. Wu., Enhanced Phytoremediation of Salt-Impacted Soils Using Plant Growth-Promoting Rhizobacteria (PGPR). *Master of Science Thesis to the University of Waterloo, Waterloo, Ontario, Canada.*, 2009; 1-155.