BIOPROSPECTING OF RHIZOSPHERIC BACTERIA ASSOCIATED WITH COASTAL SAND DUNE PLANTS FOR THE PRODUCTION OF PHYTOHORMONE

M. Jayaprakashvel and V. Ramabhai
Department of Marine Biotechnology, AMET Deemed to be University, Kanathur, Chennai - 603112, Tamil Nadu, India.

Abstract: Rhizobacteria associated with the Coastal sand dune plants produce biologically active metabolites to adapt the stressed environment. Importantly the plant growth promoting metabolites produced under this nutrition limited ecosystem by certain rhizobacteria lead to the sustainability of the Coastal sand dune plants. In this study, rhizospehere soil samples of three predominant coastal sand dune plant species such as *Ipomoea pes caprae*, *Canavalia rosea* and *Spinifex* sp. were collected from five different locations along Chennai coastal area. A total of 105 pure culture of rhizobacteria were isolated and given the identification codes with a prefix of AMETB. They were characterized based on their colony color, shape, texture, size and the ability to exhibit the diffusible fluorescence pigments in King's B medium. Among the 105 bacteria, 78 strains are Indole Acetic Acid (IAA) producers which were further studied for the quantification of IAA. Strains AMETB7008, AMETB7030, AMETB7041, AMETB7056 and AMETB7077 were found as effective producers of IAA, which produced 140.9µg/mL, 137.8µg/mL, 117.1µg/mL, 125.3µg/mL and 67.3µg/mL of IAA, respectively. By this study, it can be concluded that rhizospheric bacteria associated with Coastal sand dune plants in the East Coast of Tamil Nadu could produce phytohormone IAA which can be further developed as an effective plant growth promoter for agricultural applications in coastal saline soils.

***Key words*- Rhizobacteria, Coastal sand dune plants, phytohormone, salt stress, bioprospecting**

I. INTRODUCTION

Coastal Sand Dunes (CDS) are considered as one of the least explored marine ecosystem for the beneficial microbial activity. Their wide diversity serves as an ecological niche between marine and terrestrial system (Arun et al., 1999; Jayaprakashvel et al., 2014a). Due to the limitations in the nutritional composition and existence of various stressful environmental factors in the Coastal sand dunes, plants tend to rely on the microorganisms for their survival. Besides, microorganisms in this stressful environment are thought to be highly competitive in nature and thus, exploring this ecosystem for beneficial microorganisms could be much more promising. Efforts were made before to explore the beneficial bioactivities of bacteria associated with coastal sand dune plant rhizosphere with reference to East coast of India. Beneficial bacteria from the coastal sand dune plants have been reported to have plant growth beneficial bioactivities, ability to produce extracellular enzymes and also to enhance the plant growth (Jayaprakashvel et al., 2014b), bacteria can be screened widely for their beneficial bioactivities such as industrial enzymes and plant growth promoters (Jayaprakashvel et al., 2010; 2014a &b; Muthezhilan et al., 2012).

Coastal sand dune rhizobacteria are capable of producing various plant growth promoting bioactivities such as phosphate solubilization, ACC deaminase activity, production of IAA, siderophores and HCN (Godinho et al, 2010). *Pseudomonas* sp., AMET1055 isolated from Coastal sand dune vegetation found to have hydrogen cyanide mediated antagonistic activity against *Rhizoctonia solani*, causative agent of rice sheath blight (Jayaprakashvel et al., 2010). Hence by the continuous exploration of the rhizobacteria from the coastal area of East coast, Tamil Nadu, along Bay of Bengal, India, many useful bioactivities can be utilized. Muthezhilan et al. (2012) have isolated two coastal sand dune rhizobacteria from *Ipomoes* spp.in the Chennai coastal area. Those two bacterial strains, AMET1136 and AMET1148 exhibited excellent growth inside the seed germination of black gram and green gram. They were also demonstrated to produce IAA and solubilize tri-calcium phosphate.

Previous studies have indicated that rhizosphere bacteria could produce phytohormones. Hence, the present study has aimed to isolate and select efficient rhizobacteria that could be used as bioinoculants for the promotion of plant growth in coastal saline soils. As a preliminary report, this article provides information of the rhizobacteria that are selected based on their ability to produce high quantities of Indole Acetic Acid.

ABBREVIATION

CDS	Coastal Sand Dunes
IAA	Indole Acetic Acid
ACC	1-Aminocyclopropane-1-Carboxylate
HCN	Hydrogen Cyanide Production
OD	Optical Density
LB	Luria-Bertani
UV- VIS	Ultra Violet visible

II. MATERIALS AND METHODS

2.1 Collection of rhizosphere samples of coastal sand dune plants

Soil samples were collected from the rhizosphere soil of coastal sand dune vegetation at different locations in Chennai Coastal area by following soil biological methods as described by Sorensen et al., (2008). Usually the rhizosphere soil can be collected by tapping the soil from the roots, but the soil making up the sand dune is loose hence the roots are collected along with the soil. The roots and soil were collected using sterile forceps and spatula. The collected sample was kept in a sterile cover for further processing. A total of 24 rhizosphere soil samples were collected from five different locations along Chennai Coastal Area during November 2017 like Ennore (13.2146° N, 80.3203° E), Adyar (13.0012° N, 80.2565° E), Injambakkam (12.9198° N, 80.2511° E), Uthandi (12.8691° N, 80.2447° E) and Vadanemmeli (12.7260° N, 80.1893° E). In these locations, three plants such as *Ipomoea pes caprae, Canavalia rosea* and *Spinifex* sp. were found predominant. Identification of coastal sand dune plants in the east coast of Tamil Nadu was done using standard methods. The Plant materials were brought to the laboratory for identification. The samples were identified by using stranded reference manual (Cappers and Bekker, 2013).

2.2 Isolation of rhizosphere bacteria

Serial dilution of rhizosphere samples were prepared by suspending 10 g of rhizosphere soil in 90 ml of sterile sea water. The flasks were incubated in an orbital shaker at 150 rpm for 1 h. They were serially diluted up to 10^{-9} dilutions. The 100 µl aliquots from different dilutions were transferred and spread onto nutrient agar plates. In all cultivation experiments, the agar plates were incubated for 2 days at 35˚C. Based on the colony characteristics, single colonies were selected and stored in 15% glycerol at -20˚C for later characterization and identification.

2.3 Growth Characteristics of rhizobacteria from Coastal sand dune plants

All the 105 pure cultures of rhizobacteria were given identification codes with a prefix of AMETB. They were grown in Nutrient Agar prepared in aged seawater and their growth characteristics such as colony colour, shape, texture and size were recorded. Further their ability to produce diffusible fluorescent pigments in Kings B Medium was also recorded.

2.4 Detection of IAA production

Selected strains were tested for IAA production by Brick et al., (1991). The bacterial strains were left to grow in LB broth for 2 days at 35˚C, in the incubator. It was then centrifuged at 10,000 rpm for 10 mins and the supernatant became accumulated. To 2ml of the supernatant, 2 drops of ortho phosphoric acid become delivered and incubated at 37° c±2 for 10 minutes, and then to it 4 ml of salwolksi reagent was added. Development of pink color indicates the presence of IAA and no color change indicates the absence of IAA.

2.5 Quantification of IAA

24h grown liquid cultures (grown in LB broth) of selected bacteria have been centrifuged at 10,000 rpm for 10 minutes and the supernatant was separated. To two ml of it, three ml of salwoski reagent was brought and incubated for half-hour. A blank containing the reagent and water was used for calibration in UV Vis Spectrophotometer. The test samples were read at 530nm (OD) using IAA standard graph (Ahmad et al., 2005). Higher OD value indicates higher concentration of IAA. The quantification of IAA was done by plotting the OD values of test samples against a standard graph of IAA.

III. RESULTS AND DISCUSSION

Plant growth promoting rhizobacteria (PGPR) which includes bacterial strains from the genera such as *Agrobacterium, Bacillus, Azospirillum, Burkholderia, Erwinia* and *Rhizobium* were long been used as bioinoculants for the enhancement of plant growth (Dursun et al., 2008). PGPR was reported to enhance the plant growth by various direct and indirect mechanisms. They are found to improve the plant growth directly by producing phytohormones such as IAA, ABA etc. They also found to help the plants in improving nutritional uptakes by solubilizing and mobilizing minerals such Phosphorus, Potassium, Zinc and Silica. They also reported to help in iron nutrient mobilization through the production of siderophores. They are also found to enhance the plant growth indirectly by antagonizing the pathogenic microorganisms through the production of cell wall lytic enzymes, antibiotics and volatile metabolites (Glick, 1995; Jeon, 2003; Joo, 2005; Lee, 2005; Lucy, 2004; Ryu, 2005; Ryu, 2006; Selosse, 2004; Sessitsch, 2004).

A total of 24 rhizosphere soil samples of three plant species such as *Ipomoea pes caprae, Canavalia rosea and Spinifex sp.,* were collected from five different locations along Chennai Coastal Area (Table 1). The collected samples were screened for the rhizobacterial isolation and cultures were made with the different aliquots of the sample dilution and were spread on nutrient agar plate. The plates were incubated for two days at 35°C. From the 24 rhizophere soil samples, a total of 105 rhizobacteria with distinct colony morphology were isolated in pure culture form (Table 2 and Figure 1). They were stored as glycerol stock at -20°C. All the 105 bacteria were characterized for their growth characteristics in NA medium. From the experimental studies it was observed that among 105, only 7 rhizobacteria were able to produce diffusible fluorescent pigments in Kings B Medium. This indicates that the fluorescent *Pseudomonads*, one of the most dominant colonizers of rhizosphere are found very less in number in coastal sand dune plants may be due to the harsh environmental conditions. During the process of screening, Salwoski reagent was used to detect the presence of IAA. The indole ring in IAA produces pink color with Salwoski reagent. Based on the change of color from yellow to pink, the presence of Indole acetic acid was confirmed. The values of the various strains along with Salwoski reagent was checked using UV-VIS spectrophotometer and their values were noted down in terms of optical density.

From these OD values using a standard graph, the values were converted to µg/ml. The best results in each category were observed shown in AMETB7008, AMETB7030, AMETB7041, AMETB7056, and AMETB7077 with a production of 140.9µg/ml, 137.8µg/ml, 117.1µg/ml, 328.5µg/ml and 67.3µg/ml respectively (Figure 2).

It has been estimated that a total of 6744968 ha soil in India is affected by salt (includes saline and sodic soils) of which 1246136 ha are coastal saline soils (Arora et al., 2017). Excess salinity in soils reduces the growth and yield of crop plants. Food and Agriculture Organization of the United Nations has prioritized that specific approaches are needed for their reclamation and management to maintain their long term productivity in these saline soils (Arora et al., 2017). Hence the reported work has emphasized on selecting suitable inoculants for the alleviation of salt stress in the coastal saline soils by the use of rhizobacteria bacteria from the marine environment. These beneficial microorganisms especially enodphytic and rhizospheric bacteria are capable of exerting beneficial bioactivities for the promotion of plant growth and plant protection.

Table 1: Locations and number of rhizosphere soil samples collected

Location	**Available plant species**	**Number of rhizopshere samples collected**
Ennore	*Ipmoea pes caprae*	2
Adyar	*Ipomoea pes caprae* *Canavalia rosea*	4
Injambakkam	*Ipomoea pes caprae* *Canavalia rosea* *Spinifex* sp.	6
Uthandi	*Ipomoea pes caprae* *Canavalia rosea* *Spinifex* sp.	6
Vadanemmeli	*Ipomoea pes caprae* *Canavalia rosea* *Spinifex* sp.	6
Total samples collected		**24**

Table 2: Number of rhizobacteria isolated from coastal sand dune plants

Location	**Sample Codes**	**No of rhizobacteria isolated**
Ennore	ENI1, ENI2	11
Adyar	ADI1, ADI2 ADC1, ADC2	19
Injambakkam	IJI1, IJI2 IJC1, IJC2 IJS1, IJS2	23
Uthandi	UTI1,UTI2 UTC1,UTC2 UTS1,UTS2	27
Vadanemmeli	VNI1, VNI2 VNC1,VNC2 VNS1,VNS2	25
Total		**105**

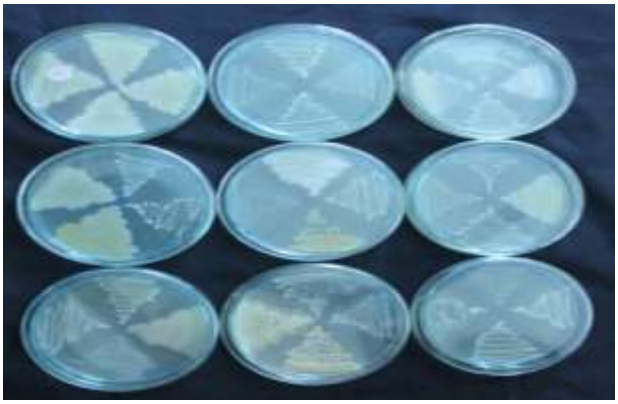

Figure 1: Pure cultures of rhizobacteria isolated from coastal sand dune plants

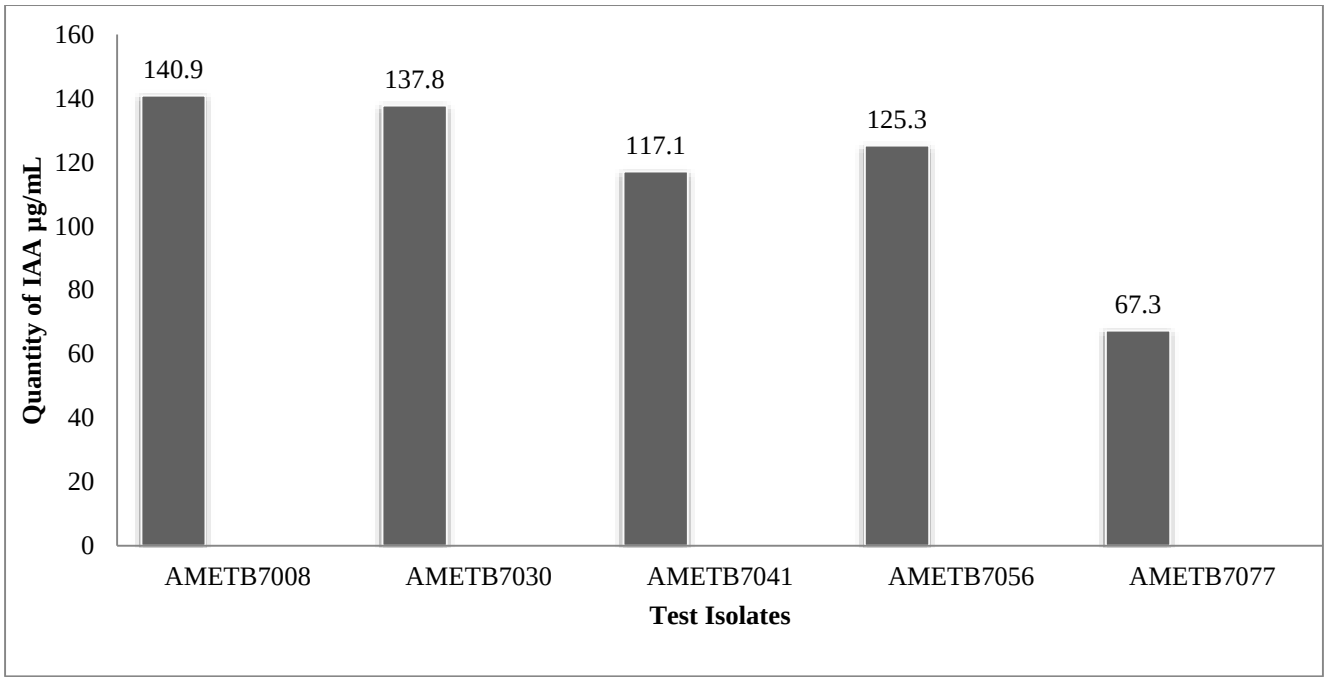

Figure 2: IAA production by selected strains: Quantitative Analysis

IV. CONCLUSION

It is concluded that these rhizobacteria associated with the plants of less studied marine ecosystem i.e. coastal sand dunes can be used as potential bioincoulants for enhancing the crop productivity in the saline soils especially coastal saline soils of India.

V. ACKNOWLEDGEMENT

Authors are thankful to the Administration and Management of AMET deemed to be University, Kanathur, Chennai, for their continuous encouragement and constant support. Author VR acknowledges the financial support provided by AMET deemed to be University.

REFERENCES

1. Ahmad, F., Ahmad, I. and Khan, M.S. 2005. Indole acetic acid production by indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan, Turkish Journal of Biology. 29:29-34.
2. Arora, S., Singh, A. K. and Singh, Y.P. 2017. Bioremediation of Salt Affected Soils: An Indian Perspective. Springer International Publishing. DOI 10.1007/978-3-319-48257-6_2.
3. Arun, A.B., Beena, K.R., Raviraja, N.S. and Sridhar, K.R. 1999. Coastal sand dunes: a neglected ecosystem. Current Science. 77:19–21.
4. Brick, J.M., Bostock, R.M. and Silverstone. 1991. S.E. Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane Appl. Environ. Microbiol. 57: 535-538.
5. Cappers, R.T.J. and Bekker, R.M. 2013. A Manual for the Identification of Plant Seeds and Fruits. Barkhuis, Eelde.
6. Dursun, A., Ekinci, M. and Donmez, M.F. 2008. Effects of inoculation bacteria on chemical content, yield and growth in rocket (*Eruca vesicaria* subsp. *sativa*). Asian J. Chem., 20: 3197-3202.
7. Glick, B. R. 1995. The enhancement of plant growth by free living bacteria. Can. J. Microbiol. 41: 109-117.
8. Godinho, A., Ramesh, R. and Bhosle, S. 2010. Bacteria from sand dunes of Goa promoting growth in Egg plant. World Journal of Agricultural Science. 6: 555-564.
9. Jayaprakashvel, M., Abishamala, K., Periasamy, C. M., Satheesh, J., Hussain, A. J. and Vanitha, M. C. 2014b.Isolation and Characterization of Indole Acetic Acid (IAA) Produced by a Halo Tolerant Marine Bacterium Isolated from Coastal Sand Dune Plants. Biosciences Biotechnology Research Asia. 11: 263-269.
10. Jayaprakashvel, M., Divyalakshmi, R. Venkatramani, M.Vinothini, S. Muthezhilan, R. and Jaffar Hussain, A. 2014a. Bioremedation of Industrial Effluent using Immobilized Cells of Halo-tolerant Marine Bacterium. Biosciences Biotechnology Research Asia. 11:69-79.
11. Jayaprakashvel, M., Muthezhilan, R., Srinivasan, R., JaffarHussain, A., Gopalakrishnan, S., Jacky Bhagat,Kaarthikeyan, N. and Muthulakshmi, 2010. Hydrogen cyanide mediated biocontrol potential of *Pseudomonas* sp.AMET1055 isolated from the rhizosphere of coastal sand dune vegetation. Journal of Advanced Biotechnology. 9: 39- 42.
12. Jeon, J. S., Lee, S. S., Kim, H. Y., Ahn, T. S. and Song, H. G. 2003. Plant growth promotion in soil by some inoculated microorganisms. J. Microbiol. 41: 271-276.
13. Joo, G. J., Kim, Y. M., Kim, J. T., Rhee, I. K., Kim, J. H. and Lee, I. J. 2005. Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of redpeppers. J. Microbiol. 43: 510-515.

14. Lee, Y. K., Jang, Y. S., Chang, H. H. , Hyung, S. W. and Chung, H. Y. 2005. A putative early response of antifungal Bacillus lentimorbus WJ5 against the plant pathogenic fungus, *Colletotrichum gloeosporioides*, analyzed by a DNA microarray. J. Microbiol. 43: 308-312
15. Lucy, M., Reed, E., Glick, B. R. 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek 86: 1-25.
16. Muthezhilan, R., Sindhuja, B.S., Jaffar Hussain, A. and Jayaprakashvel, M, 2012. Efficiency of Plant Growth Promoting Rhizobacteria Isolated from Sand Dunes of Chennai Coastal Area. Pakistan Journal of Biological Sciences, 15: 795-799.
17. Ryu, C. M., Kim, J. W., Choi, O. H., Park, S. Y., Park, S. H. and Park, C. S. 2005. Nature of a root-associated *Paenibacillus polymyxa* from field-grown winter barley in Korea. J. Microbiol. Biotechnol. 15: 984-991.
18. Ryu, J. H., Madhaiyan, M., Poonguzhali, S., Yim, W. J., Indiragandhi, P., Kim, K. A. , Anandham, R., Yun, J. C., Kim, K. H. and Sa, T. M. 2006. Plant growth substances produced by *Methylobacterium* spp. and their effect on tomato (Lycopersicon esculentum L.) and red pepper (*Capsicum annuum L.*) growth. J. Microbiol. Biotechnol. 16: 1622-1628.
19. Selosse, M. A., Baudoin, E. and Vandenkoornhuyse, P. 2004. Symbiotic microorganisms, a key for ecological success and protection of plants. C.R. Biol. 327: 639-648.
20. Sessitsch, A., Reiter, B. and Berg, G. 2004. Endophyticbacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. Can. J. Microbiol. 50: 239-249.
21. Sorensen, J., Jensen, L. E., and Nybroe, O. 2008. Soil and rhizosphere as habitats for *pseudomonas* inoculants: New knowledge on distribution, activity and physiological state derived from micro-scale and single-cell studies. Plant soil 232:97-108.