Isolation and Description of Plant Growth Promoting Rhizobacteria (PGPR) from Banana Rhizosphere soil and Its impact on plant growth promotion (*Vigna radiata*)

Dhivya. K [1*], Gajalakshmi. K [2], Ramesh Subramani[3]

[1]Research Scholar, Department of Botany, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu – 641004, India.

[2]Assistant Professor, Department of Botany, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu – 641004, India.

[3]Senior Scientist / In charge for Dean Research, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu - 641 004. India.

Abstract

Plant Growth Promoting Rhizobacteria (PGPR) are free living soil microorganisms that exert beneficial effects on plants. In the present study bacterial strains were isolated from Banana rhizosphere soil. These strains were characterized based on morphological and biochemical studies and identified as *Bacillus* spp. From the isolated Bacterial strain their Plant Growth Promoting Hormones such as IAA production, GA production and Phosphate solubilization activity was analysed. The culture filtrate of this bacterium was bio assayed on *Vigna radiata* and found that it significantly promotes the growth of the plant.

Key words: PGPR, Banana rhizosphere soil, *Bacillus* spp, Plant Growth Promoting Hormones, *Vigna radiata*.

1. Introduction:

Soil is an essential portion of the natural surrounding and is obligatory for the nourishment of life. Soil is composed of minerals and organic matter which holds the nutrients, while soil and water makes it available to plants (Patel *et al*. 2015). The layer of soil influenced by plant root (Saharan and Nehra 2011), is known as Rhizosphere that play an essential role in plant growth and development (Hrynkiewicz and Baum 2012). Rhizobacteria antagonistically colonize roots of plants, able to multiply and survive in the presence of an opposing microflora (Antoun and Kloepper, 2001). The rhizobacteria in the rhizosphere can be neutral, harmful or beneficial for plant growth. Around 2 to 5% of rhizobacteria, when reintroduced in rhizosphere, have beneficial effect on plant growth and are termed as plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). The well-known genera of PGPR are *Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella*, and *Pseudomonas.*

The means by which PGPR enhance the nutrient status of host plants can be categorized into five areas: (1) biological nitrogen fixation, (2) increasing the availability of nutrients in the rhizosphere, (3) inducing root surface area, (4) enhancing other beneficial symbiosis of the host, and (5) combination of modes of action (A.R.Apastambh, K.Tanveer 2006). There are several PGPR inoculants currently commercialized that seem to promote growth. The use of PGPR inoculants as biofertilizers and/or antagonists of phytopathogens provide a promising alternative to chemical fertilizers and pesticides. They are commonly used in agriculture, horticulture (Bandelier and Renaud, 1997)

Bacteria are abundantly present in the soil, interact with plant roots in the rhizosphere and enhance plant growth and development in certain instances. The plant growth promoting rhizobacteria (PGPR) develop a mutualistic relationship with the host

plants and gives a benefit to them through N2 fixation by nitrogenase, nitrate reductase activity, siderophore production, and phytohormone secretion in the rhizosphere (Fulchieri *et al.*, 1993; Cassán *et al.*, 2001a, 2001b).

According to numerous studies, PGPR include different bacterial genera. Among the PGPR used in the inoculation of plants and have given significant results, isolates belonging to Rhizobium (Afzal and Bano, 2008), Bacillus (Orhan *et al.*, 2006), Pseudomonas (Naiman *et al.*, 2009) Enterobacter (Morales-García *et al.*, 2011), Serratia (Nico *et al.*, 2012) and Pantoea (Khalimi *et al.*, 2012).

The banana plant (*Musa* sp.) has adventitious and horizontal roots proliferating the topsoil and cannot get water and nutrients from the deeper soil profile unlike other fruit crops. The undeveloped root system inhibits the utilization of essential mineral nutrients thus limiting the large-scale production of bananas under adverse tropical soil conditions. In the recent past there has been an increasing interest in soil microorganisms due to their importance in maintaining soil fertility since it has been shown that plant growth may be stimulated by vitamin-related and pathogen suppressing phytohormones produced by rhizosphere bacteria (Mia *et al.*, 2010).

The banana rhizosphere may harbour a wide diversity of PGPR that may not only aid in beneficial symbiotic relationships but may stimulate the plant growth by suppressing pathogenic organisms. Biofertilizers are widely accepted as a source of fertilizers with significant increase in crop yields (Vessey, 2003).

The development of biofertilizer composed by these phytobenefic rhizobacteria could minimize or even replace the use of chemical fertilizers while assuring a sustainable agriculture and maintaining environmental quality. For this purpose, the present work is the first study that focuses on the isolation of bacteria from the rhizosphere of banana cultured, and the selection of strains that are characterized in vitro by several positive activities for plants.

2. MATERIALS AND METHODS:

2.1 Isolation of Rhizobacteria

Rhizosphere soil samples were collected from banana plants. Plants were selected from agriculture fields showing good, healthy plant growth. Plants were carefully uprooted from the soil so that the roots and the attached soil were removed intact. Thereafter, roots with the advocate soil were transferred to sterile sample collection bags and packed for transport to the lab. Soil samples (1 g) as described above were mixed in 100 ml sterile distilled water and shaken for 20 min to get the rhizosphere suspension. Add 1ml of the soil solution to 9ml of distilled water and continued for 10 folds. 1ml of serial diluted sample was taken and poured into sterile Petri plate containing Nutrient Agar medium from 10^{-1} to 10^{-9}. The plates were incubated at 30°C for 24 h for isolation of rhizobacteria. Morphologically distinct bacterial colonies from each plate were purified by repeated sub - culturing and maintained on Nutrient agar media and stored at 4 C until used.

2.2 Morphological and biochemical characterization of isolates

Morphological and cultural characterization was done on the basis of colony size, shape, colour, margin, opacity, consistency, elevation, motility and gram staining, staining and selection of representative isolates was done. Biochemical tests performed were oxidase, amylase, gelatinase and catalase like enzyme production, citrate utilization, indole test, Vogus Proskauer test, methyl red test, H2S production, sugars (Glucose, Sucrose, Lactose, Xylose and Mannitol) fermentation, Triple sugar iron (TSI) test, nitrate reduction, urease test etc. [9].

2.3 Determination of Indole Acetic Acid

Isolates were inoculated in 100 ml King's B broth supplemented 0.1mg/ml tryptophan and incubated at 27 ± 2 °C for 4 days. Supernatant was centrifuged, acidified to pH 2.5 and extracted with 10 ml of ethyl acetate. Ethyl acetate fraction was evaporated at room temperature and residue was suspended in 2 ml ethanol and mixed with Fe-HClO4 reagent. The absorbance was measured at 530nm after 25 min (Gordon and Weber, 1951).

2.5 Estimation of GA

Twenty-five ml of the culture filtrate was taken in a test tube to which two ml of zinc acetate was added. After two minutes, 2 ml of potassium ferrocyanide was added and centrifuged at 1000 rpm for 15 minutes. To five ml of this supernatant,

five ml of 30 per cent HCl was added and incubated at 200 C for 75 minutes. The blank sample was treated with five per cent HCl and the absorbance of the samples as well as blank was measured at 254 nm in a UV-vis spectrophotometer. The amount of GA present in the extract was calculated from the standard curve and expressed as µg/ml of the medium. The standard curves of IAA and GA were prepared by using graded concentrations of IAA and GA. (Paleg, 1965).

2.6 Plant growth promoting capacity of microbial isolate:

Seeds of *Vigna radiata* were surface sterilized with 0.1% aqueous solution of mercuric chloride (Mineo, 1990). Seeds were germinated in sterilized petri dishes lined with moistened cotton. Samples were extracted with equal volume of ethyl acetate. Upper aqueous layer was taken and allowed to evaporate at room temperature and extracted metabolites were dissolved in distilled water. 15 ml of this was added to petri dishes containing 10 seeds of *Vigna radiata* length of root and length of shoot parameters (Tam and Tiquia, 1994) and bio chemical parameters of plant includes protein, carbohydrate and chlorophyll were observed.

3. Results and Discussion

3.1 Morphological and biochemical characterization of isolates:

On the basis of morphological and biochemical characters the selected isolates were identified as *Bacillus subtilis, Bacillus cereus*. (Table:1 and Table:1.1)

Table.1 Morphological Characters of the Isolates

S.no	Isolate name	Gram nature	Morphology	Motility	Endospore
1	*Bacillus subtilis*	Gram negative	Rod shaped	Motile	-
2	*Bacillus cereus*	Gram negative	Rod shaped	Motile	-

Table:1.1 Bio chemical characters of the isolates

S.no	Bio chemical test	*Bacillus subtilis*	*Bacillus cereus*
1	Oxidase test	+	+
2	Amylase test	+	+
3	Gelatinase liquification test	+	+
4	Catalase test	+	+
5	indole test	+	+
6	methyl red test	+	+
7	Vogus Proskaur test	+	+
8	citrate utilization	+	+
9	H2S production	+	+
10	Glucose	+	+
11	Sucrose	+	+
12	Xylose	+	+

13	Mannitol	+	+
14	Lactose	+	+
15	Triple sugar iron (TSI) test	+	+
16	nitrate reduction	+	+
17	Urease test	+	+

3.2 Plant Growth Promoting Traits of Isolates

IAA Production

All the bacterial isolates were positive for IAA production. IAA production ranged from 65 µg/ml to 68 µg/ml. (Table 2).

GA Production:

All the bacterial isolates were positive for GA production.GA production ranged from 98 µg/ml to 105 µg/ml. *Bacillus subtilis* was the highest producer of GA(105 µg/ml).All the isolates of *Bacillus* were positive for GA production (Table 2).

Table.2 Plant Growth Promoting Traits of Isolates

S.no	Isolate code	IAA production	GA Production	Quantitative IAA Production (µg/ml)	Quantitative GA Production (µg/ml)	Phosphate solubilization
1	*Bacillus subtilis*	+	+	**65**	**105**	+
2	*Bacillus cereus*	+	+	**68**	**98**	+

3.3 Plant growth parameters of *(Vigna radiata*)

The microbial isolates were bio assayed on *Vigna radiata* seeds for its growth promoting capacity in terms of root and shoot length of crop plants. Length of shoot and root was measured after 10 days of incubation. Results of shoot and root development is shown in (Table: 3). The plant growth (*Vigna radiata)* was considerably high compared with the control. shows higher growth performance of *Bacillus subtilis* greater than compared with *bacillus cereus.* Many bacterial isolates were reported earlier showing plant growth promoting activities and supports our findings. Ambawade and Pathade (2013) obtained 0.24 mg/ml of GA production by Bacillus siamensis BE76 isolated from banana. Similarly, many other plant growths promoting bacteria were isolated showing potential of their use in agriculture and other areas of plant research (Damam et al., 2016; Pawar *et al.*, 2016).

Table :3 Plant growth parameters of *(Vigna radiata*)

parameters	Shoot length	Root length	Leaf breadth	Leaf length	Length of the plant
Control	14.6±0.86*	2.3±0.43	0.96±0.06	3.03±0.28	16.8±0.66
Bacillus cereus	16.5±1.01	1.3±0.26	1.03±0.08	3.63±0.27	18.2±0.47
Bacillus subtilis	18.1±0.92	2.3±0.05	2.23±0.99	4.06±0.29	20.2±0.86

*Results are means ± S.E (n=3)

3.4 Bio chemical test of *Vigna radiata*

The total chlorophyll contents level of bacterial culture treated plants was shows significantly higher than the untreated plants. The similar results were observed in carbohydrates and protein content (Table4).

Table:4 bio chemical test of *Vigna radiata*

Sample	Control	*Bacillus subtilis*	*Bacillus cereus*
Carbohydrates	0.432	0.957	0.598
Proteins	0.146	0.920	0.747
Chlorophyll content	0.532	1.151	0.743

4. CONCLUSION

From this study it could be concluded, bacterial isolates were identified as *Bacillus* Spp. had potential of Growth Hormone production and can be further explored for its utilization for plant growth promoting capacity. Thus, microbial sources could be explored and applied for large scale production which is found to be economically important in many fields of plant generation like horticulture, ornamental developmental of plants etc. Their morphological parameters such as Number of leaves, length of leaves, breath of leaves, length of plants, shoot length, root length and Total length of plant showed significant improvements. The effect was also observed in the bio-chemical parameter such as carbohydrate content, protein content and chlorophyll content. Hence, results prove that plants treated with isolated bacterial culture (*Bacillus* sp) showed better growth in both morphology as well as biochemical parameters.

Acknowledgement

We are thankful to The Tamil Nadu state council for Science and Technology (TNSCST) and PSGR Krishnammal college for women, Coimbatore for their continuous support and encouragement.

References:

[1] Afzal, A., Bano, A. 2008. Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). Int. J. Agri. Bio., 10: 85 88.

[2] Ambawade MS and Pathade GR, 2013. Production of gibberellic acid by *Bacillus siamensis* BE 76 isolated from banana plant (*Musa* spp.). Int. J. Sci. Res., 4(7):394-398.

[3] Antoun, H., and Kloepper, J. W., 2001. Plant growth-promoting rhizobacteria (PGPR), Encyclopedia of Genetics, Brenner, S. and Miller, J.H., eds., Academic Press, N.Y., pp.1477-1480.

[4] A.R.Apastambh, K.Tanveer and M.M.V. Baig. Isolation and Characterization of Plant Growth Promoting Rhizobacteria from Banana Rhizosphere. International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 5 Number 2(2016) pp. 59-65.

[5] Bandelier S, Renaud R. Production of gibberellic acid by fed-batch solid state fermentation in an aseptic pilot scale reactor. Process in Biochemistry. 1997; 32:141.

[6] Damam M, Kaloori K, Gaddam B and Kausar R, 2016. Plant growth promoting substances (Phytohormones) produced by rhizobacterial strains isolated from the rhizosphere of medicinal

plants. Int. J. Pharm. Sci. Rev. Res., 37(1):130-136.

[7] Fulchieri M, Lucangeli C, Bottini R 1993, Inoculation with *Azospirillum lipoferum* affects growth and gibberellin status of corn seedling roots. Plant Cell Physiol 34:1305 1309.

[8] Gordon S.A. and Weber R.P. 1951. Colorimetric estimation of indoleacetic acid. Plant Physiology. 26: 192-195.

[9] Hrynkiewicz K., Baum C. 2012 The potential of rhizosphere microorganisms to promote the plant growth in disturbed soils (In: Environmental protection strategies for sustainable development, strategies for sustainability, Eds: A. Malik, E. Grohmann) — Springer, London-New York, pp. 35–64.

[10] Khalimi, K., Suprapta, D.N., Nitta, Y., 2012. Effect of Pantoeaglomerans on growth promotion and yield of rice. Agric. Sci. Res. J., 2(5): 240 249.

[11] Kloepper J. W. and Schroth M. N. 1978 Plant growth promoting rhizobacteria on radishes. In Proceedings of the 4th International Conference on Plant Pathogenic Bacteria. ed. Station de Pathologic Vegetal et Phytobacteriologic. Vol. 2, pp. 879-882. Angers, France

[12] Leeman M, van Pelt JA, den Ouden FM, Heinsbroek M, Bakker PAHM, Schippers B. Induction of systemic resistance against Fusarium wilt of radish by lipopolysaccharide of Pseudomonas fluorescens. Phytopathology.1995;85 :1021–1027.

[13] Mia BMA, Shamsuddin ZH, Mahmood M. 2010. Use of Plant Growth promoting Bacteria in Banana: A New Insight for Sustainable Banana Production. International Journal of Agricultural Biology 12, 459-467.

[14] Mineo L, 1990. Plant tissue culture techniques; in tested studies in laboratory teachings, Assn. Bio. Lab. Edu., 11:151-174.

[15] Morales García, Y.E., Juárez Hernández, D., Aragón Hernández, C., Mascarua Esparza, M.A., Bustillos Cristales, M.R., Fuentes Ramírez, L.E., Martínez Contreras, R.D., Muñoz Rojas, J. 2011. Growth response of maize plantlets inoculated with *Enterobacter* spp., as a model for alternative agriculture. Rev. Argent. Microbiol., 43: 287 293.

[16] Naiman, A.D., Latronico, A., Salamone, I.E.G. 2009. Inoculation of wheat with *Azospirillum brasilense* and Pseudomonas fluorescens: Impact on the production and culturable rhizosphere microflora. Eur. J. Soil Biol., 45: 44 51.

[17] Nico, M., Ribaudo, C.M., Gori, J.I., Cantore, M.L., Cura, J.A. 2012. Uptake of phosphate and promotion of vegetative growth in glucose exuding rice plants (*Oryza sativa*) inoculated with plant growth promoting bacteria. Appl. Soil Ecol., 61: 190 195.

[18] Orhan, E., Esitken, A., Ercisli, S., Turan, M., Sahin, F. 2006. Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Sci. Hortic., 111: 38 43.

[19] Patel K, Goswami D, Dhandhukia P, Thakker J. 2015. Techniques to study microbial phytohormones. In: Bacterial metabolites in sustainable agroecosystem. Ahmedabad: Springer International Publishing; p. 1–27.

[20] Paleg LG, 1965. Physiological effect of gibberellins. Annual Review of Plant Physiology 16:291–322.

[21] Raaijmakers JM, Weller DM. 2001. Exploiting genotype diversity of 2,4diasctylphloroglucinol producing Pseudomonas spp. Characterization of superior root colonizing P. fluorescens strain Q8r1-96. Applied and Environ Microbiology. 67:2545–2554.

[22] Saharan and Nehra 2011. Plant Growth Promoting Rhizobacteria: A Critical Review Life Sciences and Medicine Research, Volume 2011 1-30.

[23] Vessey JK. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255, 571–586.