Characterization of Potential Plant Growth Promoting Rhizobacteria Isolated from Rhizospheric soil of Banana (*Musa paradisiaca L.*)

Gajalakshmi K. and Dhivya K.*
Department of Botany, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu – 641004, INDIA
*kdhivyaias206@gmail.com

Abstract
The use of novel PGPR as bioinoculant is an alternative sustainable agricultural practice to improve soil health, controlling soil borne pathogens, increase crop productivity and conserve biodiversity. Group of bacteria that colonize roots of plant and help in plant growth and disease suppression by various direct and indirect mechanisms is named as Plant Growth Promoting Rhizobacteria (PGPR).

The present investigation was carried out to isolate, screen and characterize the PGPR from the rhizosphere soil of banana. Four bacterial strains were isolated from Banana rhizosphere. These strains were characterized morphologically and biochemically and studied for their plant growth promoting activities such as IAA production, GA production, Phosphate solubilisation and biocontrol traits of the isolates such as siderophore production and HCN production.

Keywords: PGPR, bio-fertilizer, pathogens, morphological, biochemical, biocontrol.

Introduction

PGPR stimulate plant growth through one or more mechanisms, either directly by supplying plant to phytohormones, phosphate solubilization, nitrogen fixation and siderophores production or indirectly protecting plant from phytopathogens through antagonistic mechanisms or generating Induced systemic resistance (ISR) in host plants. Induced resistance is a Physiological "state of enhanced defensive capacity" elicited by plant growth promoting rhizobacteria (PGPR)[39]. The rhizobacteria present positive responses to physiological growth characteristics, as well as to nutritional parameters of banana plants[6,33]. Rhizosphere is the thin layer of soil that proximately surrounds the plant roots and has an abundant nutritious element because of the constant deterioration activities of the plants[22].

The region is the rich source of microbes and microbial activity and thus better known as store house of microbes. It consists of large number of microorganisms mainly bacteria known as plant-growth-promoting rhizobacteria (PGPR)[28]. Kloepper and colleagues first make known to the term PGPR in 1978[26]. PGPR is now considered as safe means of agriculture due to the increasing yield as it holds capable solution in being safe for the environment. The most important is to protect plants from chemicals that are used to kill pests and also cause harmful impact on the environment. The use of bacterial fertilizers has made substantial development in terms of growth, health and yield of plants. PGPR also support growth by reducing the phytopathogens which reduce the yield and growth.

PGPR have been commercialized as microbial bioinoculants or biofertilizers to increase crop production[1]. PGPR offers an attractive strategy for replacement and reduction of heavy application of chemical pesticides and fertilizers[3]. Hence future scenarios can be replacement of chemical fertilizers and supporting the ecosystem in terms of safety.

Use of microbial consortia in the form of biofertilizers for reducing the use of chemical fertilizers without compromising yield is presently an important feature of research in the field of agriculture, microbiology and biotechnology[21]. The PGPRs can be further explored as potential biofertilizers for various economically important crops for sustainable agriculture.

In this context, the objective of our study was to isolate and identify the potential PGPR from banana rhizosphere.

Material and Methods

Rhizospheric Soil Collection: The soil used in this study was collected from twenty different banana growing sites of Coimbatore. 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 adherent soil were passed through a 4 mm sieve to eliminate coarse rock and plant material, thoroughly mixed to ensure uniformity transferred to sterile sample collection bags and packed for transport to the lab and stored at 4°C prior to use.

Isolation of bacterial isolates: Rhizosphere bacteria were isolated and enumerated by serial dilution technique on nutrient agar (NA) plates and incubated at 28±2 ^{0}C for 72 hrs. After incubation period, NA plates were observed for morphological appearances and number of bacterial colonies. Bacterial isolates having different morphological appearance on agar plates were selected and maintained on nutrient agar slants and 50% glycerol at -80°C. Bacterial colonies were selected according to the cultural and morphological characteristics including pigment, colony form, elevation and margin; texture and opacity on their selective media[32].

Characterization of bacterial isolate for different plant growth promoting activities

Phosphate solubilisation: A loop full of fresh bacterial cultures was streaked on the centre of agar plates modified with Pikovskaya agar with insoluble tricalcium phosphate (TCP) and incubated for 120 h at 28±2°C[25]. The presence of halo zone around the bacterial colonies indicated positive phosphate solubilization ability. The solubilization zone surrounding the developed bacterial colony was calculated as phosphate solubilisation index (PSI).

PSI = A/B×100

where A = total diameter of colony and halo zone and B = Colony diameter.

Production of indole acetic acid (IAA): Indole acetic acid (IAA) production was quantitatively estimated by Salkowski method[8]. Bacterial cultures were grown on Nutrient broth liquid medium at 36±2 °C. The cultures in the flask showing dense milky white growth were tested for purity. Fifty millilitre of Nutrient (NA) broth containing 0.1% DL tryptophan were inoculated with 500 µl of 24 h old bacterial cultures and incubated in refrigerated incubator shaker at 30±0.1°C at 180 rpm for 48 h in dark. Fully grown bacterial cultures were centrifuged at 10,000 rpm for 10 min at 4°C.

Estimation of IAA production in the supernatants was done using colorimetric assay. One millilitre (1 ml) of supernatant was mixed with 100 ml of 10 mM orthophosphoric acid and 2 ml of the Salkowski reagent (1 ml of 0.5 M FeCl3 in 50 ml of 35% HCIO4) at 28±2ºC for 30 min. Development of pink colour in test tubes at the end of the incubation indicated IAA production[13]. Quantification of IAA was measured by the pink colour absorbance at 530 nm after 30 min in UV/Vis spectrophotometer.

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 percent 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[23].

Biocontrol Activities

HCN production: Whatmann no. 1 filter paper pads were placed on the lid of Petri plates and the plates were sterilized. TSA medium amended with glycine (4.4 g/l) was sterilized and poured into the sterile plates. The isolates were streaked on the medium. The filter paper padding in each plate was soaked with 2 ml sterile picric acid solution. The plates were sealed with parafilm in order to contain gaseous metabolites produced by the antagonists and to allow for chemical reaction with picric acid present in the filter padding. After incubation for a week of time, the colour change of the filter paper was noted and the HCN production potential of the antagonists was assessed[38].

Production of siderophores: The plates of TSA were spot inoculated with test bacteria and incubated at 28 ± 2°C for 3 days. A layer of chrome azurol S medium (CAS)[31] was poured on the surface of each plate. After 24 h in the dark, development of orange halo around the bacteria was considered as positive for siderophores production.

Results and Discussion

PGPR colonize roots of plant and exert beneficial effects on plant growth and development by diverse mechanisms.[7] In the present study, PGPR were isolated from banana rhizosphere and characterized for various plant growth promoting activities. Four pure bacterial isolates were obtained from banana rhizospheric soil sample based on colony morphological and designated as B1, A8, A3, B2 (Table 1 and figure 1).

On the basis of cultural and biochemical conditions, selected isolates were identified as *Pantoea Sp* for A3, *Stayphylococcus Sp.* for B2, *Serratia Spp.* for isolate A8 and B1. The microscopic observation such as gram staining, shape and motility of bacterial isolates are illustrated in table 2.

Two isolates (A3 and A8) were found to be gram negative rod shaped and motile while two (B1 and B2) were gram positive, cocci shaped and non-motile.

Screening of bacterial isolates for multifarious plant growth promoting traits isolated from rhizospheric soil of Banana: These isolates were screened for their ability to perform diverse plant growth promoting activities i.e. P-solubilization, growth on nitrogen free medium, siderophore production, auxin, HCN production.

Table 1
Description of bacterial isolates

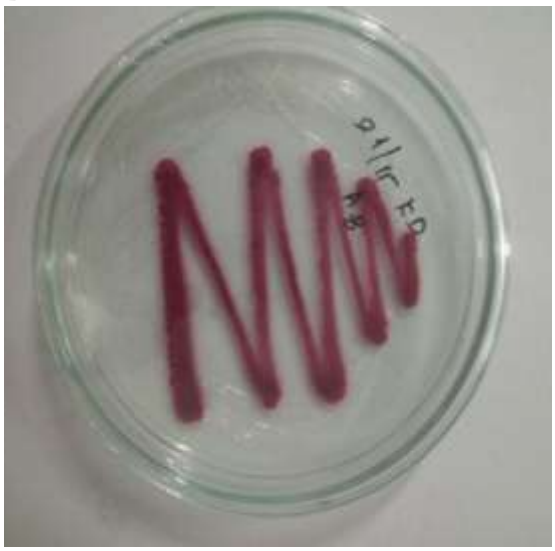
S. N.	Isolate code	Location	Soil sample
1	A3	Annur, Coimbatore	Banana Rhizospheric soil
2	B1		
3	A8		
4	B2		

Table 2
Microscopic observation of bacterial isolates

S.N.	Isolate code	Grams Nature	Shape	Motility
1	*A3(Pantoea Spp)*	-	Rod	Motile
2	*B1(Serratia Spp)*	+	Cocci	Non motile
3	A8*(Serratia Spp)*	-	Rod	Motile
4	*B2(Stayphylococcus Spp)*	+	Cocci	Non motile

B1

B2



A8

A3

Fig. 1: Morphological characters of Bacterial isolates

Phosphate solubilization: Phosphorus is the second most important nutrient, next to nitrogen (N) required for growth of plants. A greater portion of phosphorus in soil is in the form of insoluble phosphates and cannot be used directly by the plants.

In the present study, isolated four bacterial strains were found to give clear zone on Pikovskaya agar containing insoluble mineral phosphate such as tri-calcium phosphate (Table 3). Phosphate solubilization index was higher in B1(199.25±0.52) and least percentage in A3(145.53±0.14). Moreover, this isolate was also found to be medium producer of IAA. Several studies disclosed that higher concentration of phosphate-solubilizers is usually found in the rhizosphere in comparison to bulk soil. Phosphate solubilizing bacteria convert the insoluble form of phosphorus to soluble form through acidification, secretion of organic acids or protons. Thus, P-solubilization is considered as one of the most important attributes of the PGPR[24].

IAA production: Auxin was the most efficient plant growth hormone and among them the indole acetic acid

was the most common. About 80% of rhizosphere bacteria were capable of producing indole 3-acetic acid. IAA is usually considered to be the most vital phytohormone that functions as an important signal molecule in the regulation of plant growth and development processes. Strains that produce large amounts of auxin in the soil caused a maximum increase of the growth and crop yield[18,29,34].

It has been reported that more efficient auxin producers are commonly associated with rhizosphere soil in comparison to bulk soil. IAA production was found in the range of 95 to 65 µg/ml. Among all isolates, A8 was found to produce high amount of IAA i.e. 95 µg/ml and B1 was found to produce least amount of IAA i.e. 65 µg/ml. Furthermore, production of IAA by PGPR isolates may vary from different species and strains and was additionally influenced by substrate availability, growth stage and culture conditions. According to Barazani and Friedmann[5], bacteria able to secrete a higher rate to 13.5 µg L^{-1} of indole compounds were considered as PGPR.

GA Production: Gibberellins are biologically active, endogenous hormones of higher plants that are involved in a range of developmental processes in higher plants including stem elongation, germination, seed dormancy, fruit senescence and sex expression[12]. Different gibberellins (GAs) selectively affect different parts of the plants. GA production was found in the range of 25 µg/ml to 56 µg/ml. The higher the amount of gibberellin production was found in A3 i.e. 56 µg/ml, the lowest range was observed in B2 i.e. 25 µg/ml.

Biocontrol Traits of Isolates

HCN production: One of the secondary metabolites produced by certain rhizobacteria was hydrogen cyanide. Although, this compound was a general metabolic inhibitor, it was synthesized and secreted by certain bacteria as a means to avoid predation or competition[16]. Biocontrol conferred by some PGPR is mainly due to a synergistic combination of different antagonistic metabolites[14].

Several factors have been reported to influence the rate of HCN production. Glycine has been found to be the direct precursor of microbial cyanide production and it has been found in root exudates. This volatile compound is one of effective antagonistic compounds particularly against fungi. The HCN production may help in disease suppression.[36] Selected bacteria were assessed for HCN production, which acts as an inducer of plant resistance Out of four bacterial isolates, two isolates B1 and B2 were positive for HCN production.

Siderophore production: The siderophore production of the selected bacterial isolates was compared on the basis of their zone size (mm). The siderophore production efficiency of selected bacterial isolates was confirmed using the Chromo Azuerol Sulphate (CAS) assay.

The data were presented in table 4. Siderophore production by rhizobacteria acts as biocontrol mechanism under iron limiting condition[37].

Siderophore producing microorganisms protects plants at two levels: first, limiting growth of plant pathogens and secondly triggering plants defensive mechanism[27]. Out of four bacterial isolates, two isolates B1 and A3 showed positive activity for siderophore production and it is depicted by the development of orange halos surrounding the bacterial colonies in blue agar medium.

Table 3
Screening of bacterial isolates for multifarious plant growth promoting traits isolated from rhizospheric soil of Banana

S.N.	Isolate	P- solubilization		IAA	Quantitative IAA Production (µg/ml)	GA_3	Quantitative GA Production (µg/ml)
		Halo formation	PSI (%)				
1	*A3(Pantoea Spp)*	+	145.53±0.14	+	92	+	56
2	*B1(Serratia Spp)*	+	199.25±0.52	+	65	+	42
3	A8*(Serratia Spp)*	+	179.2±0.61	+	95	+	46
4	*B2(Stayphylococcus Spp)*	+	159.16±0.50	+	72	-	25

Table 4
Biocontrol Traits of Isolates

S.N.	Isolate code	Siderophore production	HCN production
1	*A3(Pantoea Spp)*	++	-
2	*B1(Serratia Spp)*	++	+
3	A8*(Serratia Spp)*	-	-
4	*B2(Stayphylococcus Spp)*	-	+

No colour change – No HCN production (-), Complete orange - Strong HCN production (+++)

Conclusion and Recommendations

This study indicates the presence of some efficient and effective species of bacteria in Annur region of Coimbatore, Tamil Nadu, India. The results suggest that four isolates revealed plant growth promotion activities that are found to produce siderophores, IAA, GA_3, HCN. The effectiveness of PGPR isolates clearly indicates that the chemical fertilizers rate or dose could be reduced through combination of PGPR isolates with fertilizers that may be an eco-friendly and cost-effective management strategy.

These native isolates may be used as efficient bio-inoculants for integrated nutrient management in the soils facing severe threat of erosion and degradation. Therefore, these isolates might have potential in future field applications as plant growth promoters. These bacterial isolates are able to provide better nutrient flux to the plants. The future studies should be focused on the functional characterization of PGPR for practical applications in the field.

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