

Original Research Article

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Isolation and Characterization of Plant Growth Promoting Rhizobacteria from Northern Karnataka, India

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ABSTRACT

An attempt was made to isolate and characterize rhizobacteria from rhizosphere soils of different crops grown in Vijayapur, Kalaburgi and Bagalkot districts of Northern Karnataka. Ten PGPR isolates and reference strain were subjected for functional characterization viz., IAA and GA production, P and Zn solubilization. Among the ten native rhizobacterial isolates, 8 isolates and reference strain produced IAA and GA. The amount of IAA and GA produced by the different rhizobacterial isolates ranged from 10.78 to 20.17 µg/ml of broth and 0.36 to 2.69 µg/ml of broth respectively. The diameter of P and Zn solubilization by the rhizobacterial isolates ranged from 3 to 7.5 mm and 2.0 to 6.0 mm after five days of inoculation respectively. These isolates were further subjected for morphological and biochemical characterization. The isolates exhibited wide variations with respect to colony morphology. Except one isolate (PG-189), other isolates found to have rod shaped cells. Except PG-152 and PG-186, other isolates and reference strain were found to be gram negative. Endospore formation was observed only in the isolate PG-152. With respect to biochemical characterization, all the isolates and reference strain were positive for oxidase and ammonia production tests and negative for indole production and lactose fermentation.

Keywords

PGPR, IAA, GA, P-solubilizers

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Introduction

Plant growth promoting rhizobacteria (PGPR) are a group of heterogenous bacteria that colonize the plant roots and promote growth of plants. The term PGPR was first used by Kloepper and Schroth (1978) for the

microorganisms closely associated with the rhizosphere region. It is well established that, in the rhizosphere, only 2–5% of bacteria promote plant growth (Antoun and Kloepper, 2001). The rhizosphere is a microbial hot-spot for microbial interactions due to the exudates released by plant roots, which constitute the

main food source for microorganisms, leading to efficient geochemical cycling of nutrients.

PGPRs have a positive impact on crop development, either directly or indirectly. PGPR's direct promotion of plant growth entails either supplying the plant with a bacterium-synthesized compound, such as plant growth regulators, or promoting the environmental uptake of certain nutrients (Glick, 1995). The Indirect plant growth promotion happens when PGPR prevents one or more phytopathogenic organisms from having deleterious impacts. This can occur through the production of antagonistic substances or through the induction of pathogen resistance (Glick, 1995). By using one or more of these mechanisms, a specific PGPR may influence crop growth and development. PGPR, as biocontrol agents, may behave through multiple processes, irrespective of their role in promoting immediate development, such as manufacturing of auxin phytohormone, reduction of crop ethylene concentrations (Glick, 2012) or root-related nitrogen fixation (Dobereiner, 1992). PGPR and their interactions with plants are exploited commercially and hold great promise for sustainable agriculture.

The excessive use of synthetic fertilizers has contributed not only soil health degradation, but also many major environmental issues, such as soil and water contamination and other health-related problems besides increasing the input cost for crop production especially on the marginal farmers (Gupta *et al.*, 2015). This situation emphasized the need for developing alternate production systems that are ecofriendly to the environment and is more judicious in maintaining soil health. One of the ecofriendly, alternate to chemical fertilizer is the use of biofertilizers. Thus, in the present study attempts have been made to screen efficient plant growth promoting

rhizobacteria from northern regions of Karnataka.

Materials and Methods

Collection of rhizosphere soil samples and isolation of rhizobacteria

Rhizosphere soil samples were collected from predominant crops *viz.*, pigeon pea, maize, chickpea, banana etc. and placed in sterile polyethylene covers, labelled and bought to the laboratory for the isolation of rhizobacteria.

Rhizobacteria were isolated from the soil samples following serial dilution and plating technique using nutrient agar and Tryptic soya agar media. The typical bacterial colonies were selected and purified by four way streaking. The isolated colonies were transferred to nutrient agar slants and used for the study.

Functional characterization

Ten rhizobacterial isolates *viz.*, PG-145, PG-148, PG-152, PG-165, PG-178, PG-180, PG-185, PG-186, PG-189 and PG-197 were subjected for functional characteristics *viz.*, IAA and GA production, P and Zn solubilization.

IAA production

Luria Bertani broth amended with 50 µg/ml tryptophan was inoculated with 1 ml of overnight grown culture of the isolates and incubated at 28°C for 24 h on rotary shaker. Culture broth was centrifuged at 10,000 rpm for 15 minutes and 4 ml of supernatant was taken in a test tube and 2 to 3 drops of orthophosphoric acid was added. To this mixture, 2 ml of Salkowski's reagent was added and incubated for 30 minutes at room temperature until the development of pink

colour (Gordon and Webber, 1951). The pink colour developed solution was read at 535 nm in a spectrophotometer.

GA production

Twenty five ml of the fresh culture supernatant was taken in a test tube to which two ml of zinc acetate was added. After two minutes, two 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 hydrochloric acid was added and incubated at 20°C for 75 min. The blank sample was treated with 5 per cent HCl. The absorbance of the samples as well as blank was measured at 254 nm in an UV-Vis spectrophotometer (Paley, 1965). The amount of GA present in the extract was calculated from the standard curve and expressed as µg/ml of the medium.

Phosphate solubilizing ability of the isolates

The ability of the test isolates to solubilize insoluble inorganic phosphate was tested by spotting 10 µl of overnight grown cultures on Pikovskaya's agar plates and incubating at 28±2°C for 2-3 days.

The isolates which showed clear zone of solubilization of tricalcium phosphate (TCP) around the colony were noted as phosphate solubilizers. The diameter of the zone of TCP solubilization was measured and expressed in mm.

Zinc solubilization ability of the isolates

The isolates were tested for their ability to solubilize insoluble inorganic zinc using mineral salt agar medium supplemented with ZnO (0.25 %). Ten µl of overnight grown culture of test isolates were spotted on the solid medium. The isolates showing clear zone around the colony on the medium was

considered as zinc solubilizers. The diameter of the zone of solubilization was measured and expressed in mm.

Characterization of the isolates

The isolates were studied for their colony and cell morphology as per the standard procedure given by Barthalomew and Mittewar (1950). The isolates were subjected to biochemical characterization by employing the standard procedures given by Cappuccino and Sherman (1992). Different biochemical tests performed were Starch Hydrolysis, Casein hydrolysis, Gelatin hydrolysis, Production of ammonia, Lactose fermentation, Nitrate reduction, Citrate utilization, Catalase test, Ureases and Oxidase test.

Results and Discussion

Isolation of Rhizobacteria

Rhizobacterial isolates were obtained from rhizospheric soil samples collected from Vijayapur, Bagalkot and Kalaburgi districts of northern Karnataka. They were designed as PG-145, PG-148, PG-152, PG-165, PG-178, PG-180, PG-185, PG-186, PG-189 and PG-197. The details of the sample and sampling locations are furnished in Table 1.

IAA and GA production

Among the ten native rhizobacterial isolates, 8 isolates and reference strain produced IAA and GA. The amount of IAA produced by the different rhizobacterial isolates ranged from 10.78 to 20.17 µg/ml of broth. The reference strain (*Pseudomonas fluorescens*) recorded significantly higher IAA production of 20.17 µg/ml broth. Among the native isolates, PG-152 showed highest IAA production of 18.49 µg/ml followed by PG-197 (17.26 µg/ml). It is reported that eighty per cent of microorganisms isolated from the rhizosphere

of different crops are able to synthesize and release auxins as secondary metabolites (Patten and Glick, 1996). The results of present study are also in line with the findings of Goudar *et al.*, (2018) and Priyanka *et al.*, (2019) who observed IAA production in rhizobacterial isolates.

Gibberellic acid is a type of phytohormone most commonly associated with modifying plant morphology by the extension of plant tissue, particularly the stem tissue (Salisbury, 1994). The amount of GA produced by the isolates ranged from 0.36 to 2.69 µg/ml of broth. The test isolate PG-197 showed highest GA production of 2.14µg/ml, followed by PG-152 (2.04µg/ml). The reference (*Pseudomonas fluorescens*) recorded highest GA production of about 2.69 µg/ml. These results are in agreement with the findings of Priyanka *et al.*, (2017) who reported that the amount of GA production by different Fluorescent Pseudomonad isolates ranged from 0.56 to 18.52 µg/25ml of broth.

Zinc solubilization by native rhizobacterial isolates

The diameter of zinc solubilization by rhizobacterial isolates ranged from 2.0 to 6.0 mm after five days of inoculation. The maximum diameter of zone of solubilization of 6.0 mm was reported by the isolate PG-152 which was more than the reference strain (*Pseudomonas fluorescens*) (3.0mm). Other isolates *viz.*, PG-145 and PG-197 showed 5.0 and 4.0mm diameter of zone of Zn solubilization respectively. Three mm diameter of zone of Zn solubilization was recorded by two of the isolates *viz.*, PG-148 and PG-178. Other three isolates *viz.*, PG-165, PG-180 and PG-185 recorded 2mm diameter of zone of Zn solubilization. Bacteria have the capacity to solubilize insoluble source of zinc (ZnO) by the release of organic acids like caffeic acid, chlorogenic acid, ferulic acid,

cinnamic acid, syringic acid and gallic acid (Hussain *et al.*, 2015).

P solubilization by native rhizobacteria

The diameter of zone of tricalcium phosphate solubilization by the rhizobacterial isolates ranged from 3 to 7.5mm after five days of inoculation. The highest diameter of zone of P- solubilization of 7.5mm was recorded by the native isolate PG-197 followed by PG-178 strain (7.0 mm). Reference strain recorded 6.5 mm diameter of zone of P- solubilization. In other isolates *viz.*, PG-148, PG-152, PG-189 and PG-145 the diameter of zone of solubilization was 6.0, 6.2, 4.0 and 3.0mm respectively. In general, phosphate solubilizing property is due to a drop in pH, which has been associated with the secretion of low molecular weight organic acids such as gluconic, 2-ketogluconic, oxalic, citric, acetic, malic, and succinic acids (Zaidi *et al.*, 2009). Mineralization of inorganic phosphate also occurs through the synthesis of a variety of different phosphatases, catalyzing the hydrolysis of phosphoric esters.

Morphological and Biochemical characterization of native rhizobacterial isolates

The isolates *viz.*, PG-145, PG-148, PG-152, PG-165, PG-178, PG-180, PG-185, PG-186, PG-189 and PG-197 processing multifunctional activities were further subjected for morphological and biochemical characterization.

With respect to the colony morphology, three of the isolates *viz.*, PG-145, PG-148, PG-185 and reference strain (*Pseudomonas fluorescens*) formed circular while other formed irregular shaped colony. Colour of the colony was transparent in case of PG-165, PG-185 and yellowish in case of PG-178 and PG-197. Creamy white coloured colony was

formed by the other isolate PG-148 and reference strain (*Pseudomonas fluorescens*). The elevation of the colony was raised in case of PG-145, PG-165 and PG-186 whereas flat in case of PG-148, PG-152, PG-180, PG-185, PG-189 and also reference strain. Convex elevation of colony was observed in PG-178 and PG-197. Colony consistency was smooth in case of PG-148, PG-178 and PG-197. Whereas it was dry in case of PG-145, PG-152, PG-180, as well as reference strain. Undulated colony margin was observed in PG-145, PG-152, PG-165 and PG-185 where as it was entire in case of PG-148, PG-178, PG-197, PG-80, PG-186, PG-189 and also in reference strain (*Pseudomonas fluorescens*). With respect to cell morphology, except one (PG-189) isolate, all the cells were found to be rod shaped. Except PG-152 and PG-186,

other isolates and reference strain were gram negative. Endospore formation was observed only in PG-152. The results are in conformity with the findings of Karnwal (2017) who isolated five rhizobacteria from maize rhizosphere and subjected them for morphological characterization. The isolates were rod-shaped. Out of five isolates, four were Gram-negative while one was Gram-positive (AK38). The isolate AK38 was endospore former and oxidase negative. While the other four isolates were endospore negative and oxidase positive. One of the bacteria isolated from potato rhizosphere was found to be rod shaped, motile and gram negative bacteria, which was further identified as *Enterobacter cloacae* (Verma and Shahi, 2015).

Table.1 Details of sampling site and samples collected for the isolation of rhizobacteria

Sl. No	Place	District	Soil type	Crop	GPS values			Code of the isolates
					Latitude (N)	Longitude (E)	Elevation (m)	
1	Kannur	Vijayapur	Black soil	Maize	17 ⁰ 02' 960"	75 ⁰ 41' 946"	577	PG-145
2	Inchageri	Vijayapur	Red soil	Pigeon pea	17 ⁰ 25' 978"	75 ⁰ 23' 874"	538	PG-148
3	Lamanatanda	Vijayapur	Black soil	Pigeon pea	17 ⁰ 26' 775"	75 ⁰ 29' 211"	557	PG-152
4	Jigaievani	Vijayapur	Black soil	Maize	17 ⁰ 44' 395"	75 ⁰ 37' 191"	575	PG-165
5	Hadalsang	Vijayapur	Black soil	Grape	17 ⁰ 53' 108"	75 ⁰ 50' 467"	504	PG-179
6	Alagudda	Kalaburgi	Black soil	Sorghum	17 ⁰ '43 "753	76 ⁰ '91 "643	491	PG-180
7	Nalvar	Kalaburgi	Red soil	Sorghum	16 ⁰ 95' 045"	77 ⁰ 07' 627"	399	PG-185
8	Bheemanalli	Kalaburgi	Black soil	Maize	16 ⁰ 95' 38"	77 ⁰ 21' 367"	513	PG-186
9	Sokanadagi	Bagalkot	Black soil	Maize	16 ⁰ 23' 409"	75 ⁰ 56' 645"	528	PG-189
10	Kovalli	Bagalkot	Red soil	Sorghum	16 ⁰ 23' 808"	75 ⁰ 58' 536"	526	PG-197

Table.2 Functional characterization of native rhizobacterial isolates

Sl. No.	Isolates	Zone of P-solubilization (Dia in mm)	Zone of Zn-solubilization (Dia in mm)	IAA (μgml^{-1} broth)	GA ($\mu\text{g ml}^{-1}$ broth)
1	PG-145	3.0	5.0	12.00	0.42
2	PG-148	6.0	3.0	13.81	1.07
3	PG-152	6.2	6.0	18.49	2.04
4	PG-165	-	2.0	-	-
5	PG-178	7.0	3.0	17.26	1.28
6	PG-180	-	2.0	13.10	1.03
7	PG-185	-	2.0	-	-
8	PG-186	-	-	10.78	0.36
9	PG-189	4.0	-	15.11	1.22
10	PG-197	7.5	4.0	16.35	2.14
11	Reference strain(<i>P. fluorescens</i>)	6.5	3.0	20.17	2.69
S.Em \pm				0.24	0.08
CD @ 1%				0.77	0.24

Note: (-) indicates no zone of solubilization.

Table.3 Morphological characteristics of rhizobacterial isolates

Sl. No	Code of the isolates	Colony morphology					Cell morphology		
		Colony shape	Colour	Elevation	Consistency	Margin	Endospore formation	Gram reaction	Cell Shape
1	PG-145	Circular	Creamy white	Raised	Dry	Undulated	-	G-ve	Rod
2	PG-148	Circular	Creamy white	Flat	smooth	Entire	-	G-ve	Rod
3	PG-152	Irregular	Creamy white	Flat	Dry	Undulated	+	G+ve	Rod
4	PG-165	Irregular	Transparent	Raised	Smooth	Undulated	-	G-ve	Rod
5	PG-178	Irregular	Yellowish	Convex	Smooth	Entire	-	G-ve	Rod
6	PG-180	Irregular	Creamy white	Flat	Dry	Entire	-	G-ve	Rod
7	PG-185	Circular	Transparent	Flat	Smooth	Undulated	-	G-ve	Rod
8	PG-186	Irregular	creamy white	Raised	smooth	Entire	-	G+ve	Rod
9	PG-189	Irregular	creamy white	Flat	smooth	Entire	-	G-ve	Cocci
10	PG-197	Irregular	Yellowish	Convex	Smooth	Entire	-	G-ve	Rod
11	Reference strain(<i>P. fluorescens</i>)	Circular	Creamy white	Flat	Dry	Entire	-	G-ve	Rod

Table.4 Biochemical characteristics of rhizobacterial isolates

Sl. No	Code of the isolates	Biochemical characteristics										
		1	2	3	4	5	6	7	8	9	10	11
1	PG-145	-	-	-	+	+	+	+	+	+	+	+
2	PG-148	+	-	-	+	+	+	+	+	+	+	+
3	PG-152	+	-	-	+	+	+	+	+	+	+	+
4	PG-165	-	-	-	-	+	+	-	-	-	+	+
5	PG-178	+	-	-	+	-	+	-	-	-	+	+
6	PG-180	+	-	-	+	+	+	+	-	-	+	+
7	PG-185	+	-	-	-	+	+	+	+	+	+	+
8	PG-186	+	-	-	-	+	+	+	+	+	+	+
9	PG-189	-	-	-	+	+	+	-	-	-	+	+
10	PG-197	+	-	-	+	-	-	-	-	-	+	+
11	Reference strain (<i>P. fluorescens</i>)	+	-	-	+	+	+	+	+	+	+	+

Note:

- 1) Starch hydrolysis 2) Indole production 3) Lactose fermentation 4) Citrate utilization
 5) Catalase test 6) Casein hydrolysis 7) Gelatin hydrolysis 8) Nitrate reduction
 9) Urease test 10) Oxidase 11) Ammonia production
 +: Positive, -: Negative

All the isolates and reference strain were positive for oxidase and ammonia production tests and negative for indole production and lactose fermentation. Except PG-145, PG-165, PG-189, other isolates and reference strain (*Pseudomonas fluorescens*) showed positive for starch hydrolysis. Except PG-165, PG-185 and PG-186, other isolates and reference strain showed positive for citrate utilization. Except PG-178 and PG-197, other isolates and reference strains (*Pseudomonas fluorescens*) were positive for casein hydrolysis. Except four isolates (PG-178, PG-165, PG-189 and PG-197), other isolates and reference strain showed positive for gelatine hydrolysis. The present work is supported by the findings of Rana *et al.*, (2011) who reported that the isolates obtained from the rhizosphere soil of wheat were found to be positive for nitrate reduction, casein hydrolysis and citrate utilization. Whereas, the isolates PG-165, PG-178, PG-180 and PG-197 showed negative for nitrate reductase and urease test.

In conclusion, indiscriminate use of chemical

fertilizer and pesticides over the last few decades has not only resulted in the contamination of environment, but also reduced the soil fertility in general and soil microbial population in particular. The use of PGPR inoculants as biofertilizers is a competent approach to replace chemical fertilizers and pesticides for sustainable agriculture in India. The present study resulted in obtaining efficient rhizobacterial isolates with multifunctional properties. These isolates can be developed as a biofertilizers after conducting appropriate field trials

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