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Isolation and Characterization of Stress Tolerant Plant Growth Promoting Rhizobacteria and their Plant Growth Promoting Activities

M Eswara Raghava Kumari, A Vijaya Gopal and R Lakshmipathy
Department of Agricultural Microbiology, APGC, Lam, Guntur, A.P.

ABSTRACT

Rhizobacteria that benefit plant growth and development are called Plant Growth Promoting Rhizobacteria (PGPR). PGPR is the rhizosphere bacteria that can enhance plant growth by a wide variety of mechanisms. A lab experiment was conducted in the year of 2016-17 at the Dept. of Agricultural Microbiology. In the present study eight soil samples were collected from black gram rhizospheric soils of Ananthapur district. From those soil samples sixteen bacterial strains were isolated and identified as eight isolates were *Rhizobium* and eight isolates was *pseudomonas.* PGP characterization of these isolates was done. Out of sixteen isolates eleven isolates exhibited maximum plant growth promoting activities. Out of 11 isolates five isolates exhibited higher phosphate solubilization efficiency.

Key words: *PGPR, Rhizobium. Pseudomonas, Plant Growth Promoting Activities*

The narrow zone of soil directly surrounding the root system is referred to as rhizosphere (Walker *et al.,* 2003). Rhizosphere is centre to microbial and nutrient dynamics and describes the zone of soil surrounding roots of plant species which release organic substances. Bacteria that are present in the rhizosphere and enhance plant growth by any mechanism are referred to as Plant Growth Promoting Rhizobacteria (PGPR). Kloepper & Schroth in 1978, first defined rhizosphere competent bacteria called, Rhizobacteria, which aggressively colonize plant roots and multiply, to provide beneficial effects in growth and yield of agricultural crops both under greenhouse and field conditions.

PGPR may improve the plant growth and yield by direct or indirect mechanisms. Direct mechanisms involve asymbiotic nitrogen fixation (Boddey and Dobereiner,1995) solubilisation of minerals including phosphorusor, production of plant growth regulators such as auxinsindole-3-acetic acid (IAA), gibberellins, cytokinins or ethylene (Arshad and Frankenberger, 1993 and Glick,1995) and synthesis of inhibitors such as 1-Aminocyclopropane-1-carboxylate (ACC) deaminase, which act directly on plant itself and affect growth. Indirect mechanisms of growth promotion include production of iron sequestering siderophores (Scher and Baker, 1982) or production of antibiotics and hydrogen cyanide (Flaishman *et al*., 1996) that serve to protect the plants from soil phytopathogens.

MATERIAL AND METHODS

Soil sample collection

Soil samples were collected up to the depth of 10 to 15cm from the rhizosphere of crop plants *i.e.,* Blackgram in Ananthapur district, Andhra Pradesh. The soil adhering to the roots was collected and mixed to provide a composite soil sample and these soil samples were kept in the polythene bag, labeled and stored at 20 ºC.

Isolation of PGPR

For isolation of rhizobacteria, the method proposed by Vlassak *et al.* (1992) was followed. In this procedure 10g of soil from each soil sample was taken in a conical flask of 90 ml water. The sample was agitated for 15 minutes on a vortex and serial dilution of soil suspension was prepared. Dilutions prepared for bacteria were given below.

For *Rhizobium* spp. - 10^{-3} to 10^{-5}
For *Pseudomonas* spp. - 10^{-3} to 10^{-5}

From respective dilutions 0.1ml portion was spread on sterilized petri plates containing specific media *i.e.,* Yeast Extract Mannital Agar for *Rhizobium*, Pikovskaya's medium for *Pseudomonas*, and the petri plates were incubated at room temperatures (28 °C ± 2 °C) for 24-72 h. Two replicates were maintained for each dilution tested. The plates were examined daily up to 3 days for bacterial colonies.

Identification of PGPR

The bacterial isolates were identified by using cultural, morphological and biochemical characteristic features described in Bergey's manual of determinative bacteriology (Holt *et al.,* 1994) and stored at 4 °C on slants and maintained through sub-culturing. The isolates were characterized by Gram staining, Methyl

Red, Voges Proskauer, Citrate and starch hydrolysis following the standard methods (Cuppuccino *et al.,* 1992).

IN VITRO SCREENING OF MULTIPLE PLANT GROWTH PROMOTING ACTIVITIES OF PGPR

Phosphate Solubilization

For this test sterilized Pikovskaya's agar was poured as a thin layer on to the sterilized petri plates and incubated for 24h. After incubation the Pikovskaya's plates were spot inoculated with isolates and incubated at 28 ± 1 ºC for 4-5 days. Formations of clear zones around the colonies were considered as positive result for phosphate solubilization.

PSE (Phosphate Solubilization Efficiency) =

$$Z / C \times 100$$

Z- Clearance zone including bacterial growth
C- Colony diameter

Indole Acetic Acid (IAA) Production

Indole acetic acid production was tested according to Gorden and Weber (1951). The active culture of each test isolate was raised in 5ml respective broth tubes and incubated at determined temperature and time. After incubation these cultures were centrifuged at recommended rpm and time. Two drops of O-phosphoric acid was added to 2ml of supernatant and incubated for 30 min to develop the colour. Development of pink colour was considered as positive for IAA production.

Ammonia production

The isolates were tested for ammonia production by inoculating the isolates in to 10 ml of pre-sterilized peptone water in the test tubes. The tubes were incubated for 48-72 h at 36 ± 2 ºC. Nessler's reagent (0.5 mL) was added in each tube. Change in colour of the medium from brown to yellow colour was taken as positive test for ammonia production.

Siderophore Production

Siderophore production was estimated qualitatively. Chrome Azurol S (CAS) Agar medium (Schwyn and Neilands, 1987) for the detection of siderophores, isolates were grown in synthetic medium, containing 0.5 µM of iron and incubated for 24 h on a rotary shaker at room temperature. Chrome Azurol S (CAS) assay is used to detect the siderophores. The CAS plates were used to check the culture supernatant for the presence of siderophores. Culture supernatant was added to the wells made on the CAS agar and incubated at room temperature for 24 h. Formation of yellow to orange coloured zone around the well indicates siderophore production.

Hydrogen Cyanide Production (HCN)

The HCN production was tested by the method of Castric and Castric (1983). First respective media plates *i.e.,* YEMA (*Rhizobium*), Pikovaskaya's broth (*Pseudomonas*), Modified Aleksandrov media (K releasing bacteria), TRIS minimal medium (Zn bacteria) were prepared separately and incubated for 24h. After that, 1ml of culture of each test isolate was inoculated on respective media plates separately. A disc of whatman filter paper No.1 of the diameter equal to the petri plate size, impregnated with alkaline picric acid solution (0.5% picric acid (w/v) in 1% sodium carbonate) was placed in the upper lid of the inoculated petri plates under aseptic condition. The control plate did not receive the inoculum. The plates were incubated-upside up at 28± 2 ºC for 48-72 h. Change in colour from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

RESULTS AND DISCUSSION

Rhizosphere soil samples from blackgram plants grown in different villages of Ananthapur district were collected and used for the isolation of PGPR using specific media. The attempts yielded 16 bacterial isolates. Among them 11 isolates exhibited efficient plant growth promoting activities by screening methods.

The isolates were identified based on morphological and biochemical characteristics and were tested for their beneficial traits like ability for solubilization of insoluble inorganic phosphate and production of plant growth promoting substances. Efficient isolates selected based on the above characters were examined by *in vitro* screening methods. The results obtained were presented as follows.

Isolation and Identification of PGPR

On the basis of cultural, morphological and biochemical characteristics 8 Rhizobial and 8 *Pseudomonas* isolates were identified from eight rhizospheric soil samples as described in Bergey's Manual of Determinative Bacteriology

Table 1. Biochemical and cultural characteristics of Rhizobial and *pseudomonas* isolates from the rhizospheric soil of blackgram

Biochemical and cultural characterization	PGPR isolates
Number of isolates	Sixteen
Gram staining	Negative
Methyl Red	Negative
Voges Proskauer	Negative
Citrate utilization	Positive
Starch hydrolysis	Negative

All eight *Rhizobium* isolates were able to form clear zone of phosphate solubilization which ranged from 10-25 mm. Among the 8 isolates, KUR1 isolate recorded the highest zone of 25 mm diameter. followed by BRR1 (23 mm), KCR1 (22 mm), STR1 (19 mm), SCR1 (18 mm), VKR1 (14 mm), KGR1 (14 mm) and BVR1isolates showed minimum solubilization zone of 10 mm. Ammonia production was shown in all isolates. Out of eight isolates KUR1 isolate produces strongly (+++) and KCR1, KGR1, STR1, BRR1 isolates produced moderately (++), weakly produced (+) by SCR1, BVR1 and VKR1 isolates. Seven of 8 isolates *i.e.,* KCR1, KGR1, KUR1, SCR1, STR1, BRR1, BVR1 were positive results for IAA production of them KUR1 isolate produced moderately (++) remaining all weakly (+) produced. Among eight isolates 7 were positive for both HCN production and siderophore production. Among them KUR1, BVR1 isolates were moderately (++) produced siderophore, remaining all (KCR1, VKR1, SCR1, STR1, BRR1) weakly (+) produced. KUR1 isolate moderately (++) produced HCN and remaining all (KCR1, KGR1, VKR1, SCR1, STR1, BRR1) weakly produced (+).

Table 2. Multiple plant growth promoting activities of Rhizobial and *Pseudomonas* isolates from the rhizospheric soil of blackgram

Isolate	Phosphate solubilization			Ammonia	IAA	HCN	Siderophore production
	Zone diameter (mm)		Solubilization Efficiency (%)	Production	Production	Production	
	Solubilization zone	Culture diameter					
KCR1	22	10	220	+ +	+	+	+
KGR1	14	10	140	+ +	+	+	-
KUR1	25	10	250	+++	++	++	++
VKR1	14	12	116.6	+	-	+	+
SCR1	18	14	128.5	+	+	+	+
STR1	19	15	126.6	++	+	+	+
BRR1	23	17	135.2	+ +	+	+	+
BVR1	10	6	166.6	+	+	-	++
KCP1	27	10	270	+++	++	++	++
KGP1	13	10	130	+	+	+	+
KUP1	14	9	155	+	-	+	+
VKP1	16	10	160	+	+	+	+
SCP1	17	10	170	+	+	+	+
STP1	16	8	200	+	+	+	++
BRP1	15	7	214.2	+	+	+	+
BVP1	12	10	120	++	+	-	+

Similar results were observed with Saravanan *et al.* (2016) isolated 17 morphologically different bacterial isolates. All the isolates were screened for plant growth promoting substances. Among 17 isolates, 5, 9 and 11 showed positive results for ammonia and phosphate solubilization, 9 isolates produced indole acetic acid.

All eight *Pseudomonas* isolates were able to form clear zone of phosphate solubilization which ranged from 12-27 mm. Among the 8 isolates, KCP1 isolate recorded the highest zone of 27 mm diameter followed by SCP1(17 mm), STP1(16 mm), VKP1(16 mm), BRP1(15 mm), KUP1(14 mm), KGP1(13 mm) and BVP1isolates showed minimum solubilization zone of 12 mm. Ammonia production was shown in all isolates. Out of eight isolates KCP1 isolate produces strongly (+++) and BVP1 isolates produced moderately (++) remaining all weakly (+) produced. Seven of 8 isolates were positive results for IAA production of them KCP1 isolate produced moderately (++) remaining all weakly (+) produced. Among eight isolates 7 were positive for HCN production and all isolates were positive for siderophore production. Among them KCP1, STP1 isolates were moderately (++) produced siderophore remaining all weakly (+) produced. KCP1 isolate moderately (++) produced HCN and remaining all weakly (+) produced.

Gopinathan and Prakash (2014) carried their research work on Isolation of plant growth promoting rhizobacteria from vermicompost and effect on growth of greengram. They concluded that both *Rhizobium* and *Pseudomonas* were positive for IAA production.

Geetha *et al.* (2014) showed enhanced growth of greengram due to the production of ammonia, IAA, HCN, phosphate solubilization.

CONCLUSION

In the present study sixteen PGPR isolates were isolated from blackgram rhizosphere soils in Ananthapur district, Andhra Pradesh . Plant growth promoting characteristics of these bacterial isolates were studied and concluded that out of sixteen isolates eleven isolates exhibited maximum plant growth promoting activities. Out of 11 isolates, five isolates (KUR-1, KCR-1, KCP-1, BRP-1, STP-1) exhibited higher phosphate solubilization efficiency.

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