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Screening of *Bradyrhizobial* Isolates for Plant Growth Promoting Properties *in vitro* Conditions

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ABSTRACT

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Plant growth promoting rhizo-bacteria (PGPR) affect plant growth by producing and releasing secondary metabolites (plant growth regulators/phytohormones/biologically active substances), facilitating the availability and uptake of certain nutrients from the root environment and inhibiting plant pathogenic organisms in the rhizosphere. At the same time, plants produce root exudates containing e.g. sugars, amino acids, organic acids, vitamins, enzymes and organic or inorganic ions. Those substances in turn influence the rhizosphere microflora and also the behaviour of PGPR. In this work, I examined the potential use of legume bacteria, rhizobia as PGPRs since it has been shown that rhizobia (legume bacteria) can function as PGPR in nitrogen fixing plants. The present investigation is carried out with the objective of isolating Bradyrhizobia from different areas of soybean growing fields of Adilabad district and treating these isolates with the nitrogen fixation responsive soybean variety, for selecting the best isolate. Fourteen Rhizobial isolates were obtained from different areas, purified and authenticated based on their morphological, cultural, biochemical characters and nodulation test. All the fourteen isolates showed checked for IAA, phosphate solubilizing activity, Siderophore production and HCN production. Among all isolates SBR-8 showed positive in all PGPR activities.

Introduction

Soybean [*Glycine max* (L.) Merrill] a legume native to East Asia has made a significant contribution to the yellow revolution in India. Starting from 3000 ha in India during 1969 soybean has shown steady increase in area over the years. At present India ranks fifth in both area and production in the world and Madhya Pradesh is a leading state in both area and production in India. In Madhya Pradesh,

soybean occupies an area of 57.300 lakh ha with 61.666 lakh million tonnes production with average yield of 1076 kg ha⁻¹ during Kharif 2010-11 (Soybean Processor Association, 2011).

Together, soybean oil and protein content account for about 60% of dry weight of soybean: protein at 40% and oil at 20%. The remainder consists of 35% carbohydrate and about 5% ash.

Soybean fixes atmospheric nitrogen

biologically in its root nodules symbiotically in association with specific *rhizobia*. *Rhizobia* are soil bacteria that fix nitrogen (diazotrophy) after becoming established inside root nodules of legumes. *Rhizobia* require a plant host because they cannot independently fix nitrogen. Morphologically, they are gram negative, motile, non-speculating rods. *B. japonicum* is slow growing, nitrogen fixing bacteria that forms asymbiotic relationship with soybean. It insists the formation of nodules in the plant root system. Plant root colonizing bacteria can function as harmful, deleterious rhizobacteria (DRB) or beneficial, plant growth promoting rhizobacteria (PGPR). Rhizobacteria that inhibit plant growth have been described as deleterious rhizobacteria. Plant growth promoting rhizobacteria (PGPR) are root associated soil bacteria that can facilitate plant growth and development in two diverse ways: directly or indirectly (Glick, 1995; Glick *et al.*, 1999). The direct promotion of plant growth by PGPR generally boost up plant growth by providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment.

A number of authors have reported that inoculation with plant growth promoting rhizobacteria (PGPR) can result in increased germination and seedling emergence and modify growth and yield of various cereal and non-cereal crops (Freitas and Germida, 1992; Chen *et al.*, 1994; Matiru and Dakora, 2004; Wang *et al.*, 2007). The growth stimulation in plants by PGPR can be a direct effect of production of secondary metabolites such as auxins, IAA, cytokinins, riboflavin and vitamins (Dakora, 2003). These stimulate growth of plant organs via cell division and expansion (Campanoni *et al.*, 2003) or by improving nutrient availability. They also release organic acids, which help to make available forms of nutrients (Biswas *et al.*, 2000) and often lead to increased plant growth

through uptake of water and mineral nutrients or indirect when the rhizobia inhibits pathogens or deleterious microorganisms by producing siderophores,

Majority of Indian soils are essentially devoid of efficient *Bradyrhizobium japonicum*. Direct isolation of efficient strains from soil is difficult from the native rhizobial population. It was thought to be ideal to isolate cultures from effectively nodulated soybean plants. Local rhizobial isolates usage as inoculants were also found to be more effective. Therefore, effective rhizobia need to be identified from among local rhizobial isolates for soybean for effective symbiotic association and thereby obtaining high yields, improved soil fertility and better environment.

Though soybean cultivation has been introduced in Adilabad district, the crop has failed to establish in many places. One of the reasons for this could be due to the absence of soybean specific indigenous rhizobial strains in the soils. Hence, it was decided to isolate strains of rhizobia from soybean, purify and screen them for their ability to nodulate so that the most efficient strains could be cultured on a mass scale and supplied to farmers.

Materials and Methods

Isolation of bacterial isolates

Root nodules collected from different local varieties of soybean crop growers from Telangana by method as described by Vlassak (1992) on the selective media plates i.e. Yeast Extract Mannitol Agar (YEMA) and incubated at 30°C for a period of 24-72h.

Characterization of bacterial isolates

The isolates that were likely to be *Rhizobium* colonies were picked up from the YEMA plates and proceeded for confirmation by

studying the cultural, morphological and biochemical examination as given below.

Cultural Characterization

After incubation, cultures were studied for their colony characters such as size, shape, margin, consistency, pigmentation etc. as per Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Morphological characters

Purified cultures were further studied for their cell morphology viz., Cell shape, Cell arrangement, response to the gram stain.

Screening for plant growth promoting properties of bacterial isolates

Phosphate solubilisation

This test will be performed following spot inoculation of pure isolates on Pikovskaya's medium (Pikovskaya, 1948).

The diameters of the clearing zones around the colonies were measured.

Indole acetic acid production

IAA production will be tested in succinate broth using orthophosphoric acid as a reagent for color development (Duby and Maheswari, 2012). Pink colour of the medium and quantity of IAA production measured by the standard graph analysis.

Siderophores production

Production of siderophores will be estimated qualitatively on aqueous Ferric chloride solution for siderophores detection (Schwyn and Neilands, 1987). Orange colour hallow zone around the colonies.

Hydrocyanic acid production

Pure isolate will be tested by inoculating on succinate agar using alkaline picric acid as a reagent (Castric and Castric, 1983). Brown colour of the filter paper positive for HCN production.

Results and Discussion

Two or three healthy pink nodules were collected from each plant and surface sterilized by using 0.1% HgCl₂ and 70% ethanol as described in Material and Methods. The nodules were crushed and streaked on YEMA medium plates containing congo red dye. The colonies from each nodule were purified by streaking 2-3 times on same media. In total fourteen Rhizobial isolates were obtained from different places. These isolates were further purified and maintained on YEMA slants for further studies.

The bradyrhizobia isolates were checked based on morphological characters for purity, labeled and maintained on YEMA slants. The Rhizobial isolates were labeled as mentioned in Table 1.

Production of plant growth promoting substances

Plant growth promoting substances such as auxins, gibberellins, ethylene substances produced by plant growth promoting rhizospheric bacteria. These are directly involved in plant growth promotion. In the present study fourteen isolates were screened for indole acetic acid production for selection of efficient growth promoting bacterial strain (Table 2).

Indole acetic acid production

Effect of IAA in plants is significant and some of them are apical dominance, phototropism, gravitropism, prevention of leaves and fruit

abscission and induction of adventitious root system. Therefore IAA has profound influence on crops. Production of IAA was observed with the supplementation of L-Tryptophan @ 10 mg per liter by all fourteen bacterial isolates. The bradyrhizobial isolates SBR-4, SBR-5, SBR-6, SBR-7, SBR-8, SBR-9, SBR-10, SBR-11, SBR-13 and SBR-14 produced IAA while other isolates SBR-1, SBR-2, SBR-3 and SBR-12 did not produce IAA (Table 2).

All the bradyrhizobial isolates were tested for their plant growth promotion properties like phosphate solubilization and for biocontrol property like siderophore and HCN production also and the results are presented in Table 2.

Phosphate solubilization was studied by observing the clear zone on Pikovskya's medium. Some of the bradyrhizobial isolates showed phosphate solubilization activity on

Pikovskya's medium such as SBR-5, SBR-6, SBR-7, SBR-8, SBR-9 in Table 2.

In the present study IAA production and phosphate solubilization by PGPR isolates agreed with the earlier reports are available on PGPR strains which were isolated from wheat showed IAA production ranging from 5.5-31.0 µg mL⁻¹ (Abbasi *et al.*, 2011). Hussain and Srinivas (2013) isolated

Since siderophore production is attributed as one of the mechanisms of biocontrol activity of the plant growth promoting rhizobacteria, siderophore production was observed on 0.2% aqueous Ferric chloride solution.

Some of the bradyrhizobial isolates produced siderophores viz., SBR-1, SBR-2, SBR-3, SBR-8 and SBR-12 in Table 2.

Table.1 Bradyrhizobial isolates from different parts of Adilabad district

Place of sample collection	Bradyrhizobial isolate
Dhanor (b)	SBR-1 & SBR-2
Devapur	SBR-3
Indervelly	SBR-4 & SBR-5
Gaurapur	SBR-6 & SBR-7
Utnoor	SBR-8 & SBR-9
Muthnor	SBR-10 & SBR-11
Bersaipet	SBR-12
Salewada	SBR-13
Ponnari	SBR-14

Table.2 Cultural and physiological characterization of the bradyrhizobial isolates from the soybean growing fields of the Adilabad district

Isolate name	IAA production	Phosphate solubilization	Siderophore production	HCN production
SBR-1	-	-	+	-
SBR-2	-	-	+	-
SBR-3	-	-	+	-
SBR-4	+	-	-	+
SBR-5	+	+	-	-
SBR-6	+	+	-	+
SBR-7	+	+	-	-
SBR-8	+	++	++	+
SBR-9	+	+	-	+
SBR-10	+	-	-	+
SBR-11	+	-	-	-
SBR-12	-	-	+	-
SBR-13	+	-	-	-
SBR-14	+	-	-	+

Note: + show for positive, - shows for negative

The bradyrhizobial isolates SBR-4, SBR-6, SBR-8, SBR-9, SBR-10 and SBR-14 produced IAA while other isolates SBR-1, SBR-2, SBR-3 and SBR-12 did not produce HCN (Table 2).

Similar results were also reported by Ponmurugan and Gopi (2006) who found that phosphate solubilizing bacteria (PSB) isolated from the rhizosphere of different field crops including maize, were capable of producing auxin under in vitro conditions.

The various isolates of bradyrhizobia isolated from the soybean plant nodules collected from different regions of Adilabad district were screened under pot culture experiment with good *Bradyrhizobium* responsive soybean variety J.S-335.

The bradyrhizobial isolates were obtained and identified based on morphological, cultural and biochemical characters. They were screened for production of phosphate

solubilization, IAA and siderophore production. Pot culture experiments were conducted to study the *Bradyrhizobium* responsive soybean variety and best Bradyrhizobial isolate suitable for nitrogen fixation responsive soybean variety. The salient findings of the experiments conducted are given below:

Bradyrhizobia isolated from the soybean fields of different areas of Adilabad district were purified.

Fourteen bradyrhizobial isolates from different regions of Adilabad district were identified as *Bradyrhizobium* and nine isolates are used in further studies.

All the bradyrhizobial isolates were slow growing and alkali producers. Based on the results obtained in the present study, it can be summarized that the J.S-335 soybean variety is highly responsive to inoculation of bradyrhizobial isolate SBR-8.

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