Isolation of plant growth-promoting rhizospheric bacteria from leguminous and non-leguminous plants

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Abstract

The plant growth-promoting microbial profile differs from leguminous tonon-leguminous plants. The rhizospheric zone of plantswas reported to be enriched with microbes having plant growth-promoting rhizobacteria. The present research focuses on the isolation of microbes having plant growth-promoting(PGP) characteristics from the rhizosphere of leguminous plants (pigeon pea) and non-leguminous plants (Cauliflower). A totalof 8 isolates were studied for PGP characteristics i.e. IAA, Siderophore, phosphate solubilizing, and hydrogen cyanide. The isolates were found to be laced with plant growth-promoting characteristics. This research gives knowledge of the utilization of these isolates for plant growth promotion in agricultural practices.

Keywords:*PGPR, rhizosphere, leguminous plant, non-leguminous plant*

Introduction

The collaborative increment of environmental damage and human population can endanger the world food production in the future (Glick 2012). It is a priority to increase agricultural productivity within the next few decades. Nowadays agricultural practices are more focused on sustainable and environmental friendly approaches. The most preferred approach is transgenic plants and utilization of plant growth-promoting characteristics for the upliftment of agriculture productivity.Plant-microbe interactions are the key determinants of plant health and soil fertility. The interactions may be harmful, beneficial, or neutral to the plants. Presently the focus is more onthe exploitation of beneficial interactions of plants and microbes. With the identification of new bacterial strains with growth-promoting traits, nowadays, the use of microbial technologies is expanding rapidly in agriculture (Garcia Fraile et al., 2015).The microbes involved symbiotically with plants through direct mechanisms such as atmospheric

nitrogen fixation, phosphate solubilization, siderophore production, and secretion of plant growth-promoting hormones whereas; the indirect mechanisms include biological control of phytopathogens /deleterious microbes through antibiotic production, lytic enzymes, siderophore and HCN secretion (Vejan et al. 2016). These mechanisms remarkably improve plant health and promote growth in terms of an increase in seedling emergence, vigor index, and crop yield *(Gholami et al., 2009).* The soil zone strongly influenced by plant roots, the rhizosphere, plays an important role in regulating soil organic matter decomposition and nutrient cycling. Rhizosphere processes are major gateways for nutrients and water. Bacteria belonging to the genera Rhizobium, Mesorhizobium, Sinorhizobium, Bradyrhizobium, and Azorhizobium (collectively referred to as rhizobia) grow in the soil as free-living organisms but can also live as nitrogen-fixing symbionts inside root nodule cells of legume plants. The use of PGPR in agriculture to enhance the growth of plants via circulating the nutrients in the soil is an eco-friendly strategy to minimize the need for synthetic fertilizers as much as possible. Hence, the present work aimed to isolate, screen, and characterize the PGPR from rhizospheric soils of pigeonpeaand cauliflower plants which can be utilized in the future to increasethe growth and yield of plants (Singha et al., 2018).

Material & Method

The glassware used in the study was of high-quality grade (Borosil) and resistant to heat as well as acids. All glassware was soaked in acid, washed with regular detergent (laboline), cleaned with tap water followed by distilled water, and then allowed to dry. They were sterilized at 121 °C (wet heat sterilization) for 20 min. and stored in clean racks. Analytical-grade chemicals were used throughout the study. Media chemicals used were of Loba and Himedia, India. Assay chemicals were from Sigma Aldrich, U.S.A.

Plant selection and Collection of rhizosphericsoil

Two plant samples growing on agricultural land one belonging to a leguminous group i.e. Pigeonpea (*Cajanus Cajun*) and anothernon-leguminous plant cauliflower (*Brassica Oleracea*). Rhizospheric soilwas collected in sterile polythene bags, labeled, and stored in a mini cooler for carrying during travel. The soil sampleswere stored in a refrigerator immediately after reaching the laboratory for further analysis.

Isolation of soil bacterial strains from enrichment cultures

For the enumeration of bacterial cells, 10 grhizospheric soil was dissolved in 0.1% saline solution for serial dilution methodon nutrient agar (NA) plates. After inoculation, the plates of serial dilution were kept for incubation at 28±2 ^{0}C for 72 hrs.After 24 hours the plates were taken out, colonies were counted in CFU and single colonies were isolated, and axenic cultures of morphologically different organisms were obtained.After the 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.These cultures were subculture 2-3 times till pure culture of the respective isolates is maintainedand maintained on nutrient agar slants and 50% glycerol at -80°C. These pure cultures are kept in refrigerator for analysis of PGP characteristics.

Colony Characterization and Biochemical Test

Total 8 isolates were selected for the study of PGPRs. The colony morphology was noted by simple visualization. For the identification of microorganisms based on cell wall characteristics, the Gram staining of the culture was carried out by Gram's method. After carrying out the preliminary morphological analysis further biochemical analysis was carried out to authenticate the identification of bacteria. Bacteria were identified and classified largely based on their reactions in a series of biochemical tests. The biochemical traits that were carried out under the present investigation include catalase activity.

Catalase test

Yeast extract tryptone broth tubes, inoculated with actively growing bacterial culture(s) were incubated for 3 days at 30°C. Catalase activity was observed by adding few drops of 3 % H_2O_2 to the broth cultures, kept on the glass slides. Formation of oxygen bubbles confirms the positive result(Graham and Parker 1964).

Identification of plant growth promoting attributes of isolated rhizobacteria

Indole acetic acid (IAA) production

Indole acetic acid (IAA) production was quantitatively estimated by Salkowski method. Bacterial cultures were grown on Luria broth liquid medium at 36±2 °C. Fifty milliliter of Luria Bertani (LB) 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. One millilitre (1 ml) of supernatant was mixed with 100 ml of 10 mMorthophosphoric 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. The development of pink color in test tubes at the end of the incubation indicated IAA production. Quantification of IAA was measured by the pink color absorbance at 530 nm after 30 min in UV/Vis spectrophotometer (Gordon and Weber 1951).

Phosphate solubilizing test

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 18. The presence of clear zone around the bacterial colonies indicated positive phosphate solubilization ability (Surange et al., 1997).

Siderophore Assay

Qualitative estimation of siderophore production by the bacterial isolates was determined by adapting the method Schwyn and Neiland on chrome azurolsulphonate (CAS). Pure culture grown on 24 h in LB broth was streaked onto King's medium B (KB) agar plates. Production of siderophore was determined by the development of orange halo zone around bacterial colonies after 3 days incubation at 27°C (Schwyn and Neilands 1987).

Result and Discussion

Colony characterization of isolates

Beneficial responses due to interaction of PGPR with rhizobia on legumes have been reported previously (Lynch, 1990; Saxena & Tilak, 1994; Maier & Triplett, 1996; Okon et al., 1998; Gupta et al., 1998; Lata et al. 2000; Azcon-Aguilar & Barea, 1981; Grimes & Mount, 1984; Hicks & Loynachan, 1989).In the present study, a total of 8 cultivable rhizosphric bacteria marked as their names were isolated from the *Cajanuscajan*and *Brassica oleracea*on nutrient medium. The colony characteristics of all the isolated bacteria were analyzed and reported in

COLONY	SHAPE	MARGIN	ELEVATION	SIZE	COLOUR
	Pigeonpea				
1	IRREGULAR	UNDULATE	FLAT	MODERATE	WHITE

2	IRREGULAR	CURLED	FLAT	MODERATE	WHITE
3	IRREGULAR	UNDULATE	FLAT	MODERATE	WHITE
4	IRREGULAR	CURLED	RAISED	MODERATE	WHITE
Cauliflower					
1	IRREGULAR	CURLED	FLAT	MODERATE	WHITE
2	CIRCULAR	UNDULATE	FLAT	MODERATE	WHITE
3	IRREGULAR	CURLED	RAISED	MODERATE	WHITE
4	CIRCULAR	LOBATE	FLAT	MODERATE	WHITE

Table 1. All the colonies appeared white in color. The isolated plant growth-promoting rhizobacteria were observed to form mostly circular and irregular colonies on the agar surface.

For differentiating the isolates were done based onGram staining. Table 2 represents the shape, arrangement, and Gram staining results of the studied isolate. We found out that half isolates were Gram-positive and the rest were Gram-negativearranged in singly or in chains.

ISOLATES OF BACTERIAL ENDOPHYTES	SHAPE	ARRANGEMENT	GRAM-POSITIVE/ NEGATIVE
pigeon pea			
1	BACILLI	SINGLE	GRAM +VE
2	BACILLI	SINGLE	GRAM +VE
1	BACILLI	SINGLE	GRAM+VE
2	COCCI	IN CHAIN	GRAM-VE
cauliflower			
1	BACILLI	IN CHAIN	GRAM+VE
2	COCCI	SINGLE	GRAM-VE
1	COCCI	SINGLE	GRAM-VE
2	COCCI	SINGLE	GRAM-VE

Table 2: Colony characterization of isolated rhizobacteria

Isolation and Identification of PGPR from rhizospheric soil of selected plants

The pigeon pea (*Cajanuscajan*) is a perennial legume from the family*Fabaceae.* Since its domestication in the Indian subcontinent at least 3,500 years ago, its seeds have become a common food in Asia, Africa, and Latin America. Pigeon pea is an important legume crop of rainfed agriculture in the semiarid tropics. Pigeon peas are very drought-resistant and can be grown in areas with less than 650 mm annual rainfall.In the present study, plant growth-promoting rhizobacteria from rhizosphere soilof pigeon pea andcauliflower was isolated. A total of 8 cultivable rhizobacteria from root soil were isolated on nutrient media. Each isolate were maintained in pure culture and further tested for their biochemical characterization.

Microorganisms have their own identifying biochemical characteristics. It is important in the identification of a genus and species of bacteria. These biochemical traits include: the determination of the kind of nutrients bacteria can use, the products of its metabolism, the response to specific chemicals, and the presence of particular enzymes. Morphological and biochemical characteristics of the isolated plant growth-promoting rhizobacteria were performed to identify the isolated PGPR. Characterization based on the morphological and biochemical features of bacteria is the most practical way of identifying bacteria. The microscopic observations such as gram staining, shape, and motility of bacterial isolates are illustrated. Out of eight isolates, 4 weremotile and 4 non-motile.

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. It has been reported that more efficient auxin producers are commonly associated with rhizosphere soil.The role of IAA phytoharmone in plant growth and development is well established. Out of eight identified bacterial PGPR, two isolates were found to be positive for IAA (Table 3).
Phosphorus is the second most important nutrient, next to nitrogen (N) required for the growth of plants. A greater portion of phosphorus in soil is in the form of insoluble phosphates and cannot be used directly by plants. Siderophore, HCN, and catalase production: Siderophore production by rhizobacteria acts as a biocontrol mechanism under iron-limitingconditions. Siderophores produced by the microorganism can bind with iron and have high specificity and affinity making the iron available for other microorganisms.In our study, we have detected three isolates positive for siderophore production. In the present study, only one isolate showed phosphatase activity

and all the negative test showed catalase activity whilst Hydrogen Cyanide was absent only in six isolates and positive in all two isolates.

ISOLATES	IAA	SIDEROPHORE	PHOSPHATE SOLUBLIZING	HYDROGEN CYNIDE	CATALASE ACTIVITY
Pigeonpea					
Peg 1	-VE	-VE	-VE	-VE	-VE
Peg 2	-VE	-VE	-VE	+VE	-VE
Peg 3	-VE	-VE	-VE	+VE	-VE
Peg 4	-VE	+VE	-VE	-VE	-VE
cauliflower					
Cal 1	-VE	+VE	+VE	-VE	-VE
Cal 2	+VE	+VE	-VE	-VE	-VE
Cal 3	-VE	-VE	-VE	-VE	-VE
Cal 4	+VE	-VE	-VE	-VE	-VE

Table 3: Plant growth promoting characteristics, catalase and Hydrogen Cyanide test of isolates

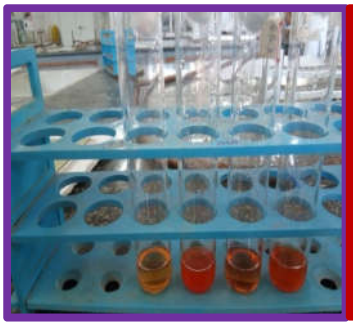

Fig.1
IAA test

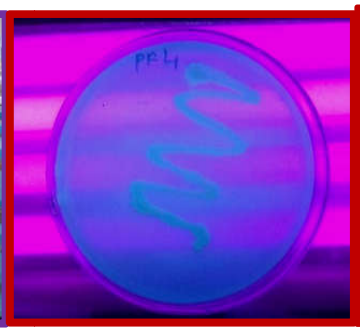

Fig. 2
Siderophore

Fig. 2
Siderophore

Conclusion

The present investigation mainly deals with isolation of PGPR from rhizosphere of Pigeon pea and cauliflower in agriculture area with special reference to their plant growth promoting traits.Total eight isolates were found from Pigeonpea (rhizospheric soil) and Cauliflower. Microbiological characterization of isolates was done by Gram staining and colony characterization.Biochemical test showed Catalase negative in all isolates.In PGPR such as IAA, Siderophore, Phosphate solubilizing and Hydrogen cyanide test was performed and pigeonpea two isolate show positive with IAA, Siderophore, Phosphate solubilizing and Hydrogen cyanide. These rhizobacteria can be further used for agricultural production enhancement, restoration of dump soil etc.

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