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RESEARCH ARTICLE

Characterization of Plant Growth Promoting Rhizobacteria associated with Potato rhizosphere.

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Abstract

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that can be found in the rhizosphere, Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. In association with roots which can enhance the growth of plant directly or indirectly. Numerous studies on PGPR has been made in all over India in several different crops but few in Chhattisgarh region and none on potato crops In the present study, four potato Rhizospheric soil samples were collected from different sites of Bilaspur C.G (India). A total of 23 bacteria were isolated. Different plant growth promoting activities i.e. phosphate solubilisation, IAA production, ammonia production, HCN production, siderophores production, amylase production, protease production and catalase production of isolated strain were carried out. Out of 23 isolates five bacterial isolates were positive for phosphate solubilisation, 15 for IAA production. Almost all the isolates were positive for ammonia production. Nine isolates were positive for HCN production, 19 for Amylase production, 14 for protease production and 16 isolates were found to be catalase positive. As PGPR are environmental friendly and offer sustainable approach to increase production of crops and health. Therefore, these isolates can be utilized for biofertilizer formulation under local agro-climatic conditions of Bilaspur (C.G), India after field trial. Which is in progress.

Introduction

Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as plant growth-promoting rhizobacteria (PGPR). In the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is a potentially important issue.
Potato is an integral part of much of the world's food supply. According to the International year of potato (2008), potato is the world's fourth- largest food crop, following maize, wheat, and rice. It is best known for its carbohydrate content (approximately 26 grams in a medium potato). In 2008, several international organizations highlighted the potato's role in world food production, in the face of developing economic problems. They cited its potential derived from its status as a cheap and plentiful crop that grows in a wide variety of climates and locales (Wade, 2008). The potato contains vitamins and minerals, as well as an assortment of phytochemicals, such as carotenoids and natural phenols. Chlorogenic acid constitutes up to 90% of the potato tuber natural phenols. Others found in potatoes are 4-O-caffeoylquinic acid (crypto-chlorogenic acid), 5-O-caffeoylquinic (neo-chlorogenic acid),

3,4-dicaffeoylquinic and 3,5-dicaffeoylquinic acids (Ferretti, 2011). Recently, there is a growing interest in PGPR due to their efficacy as biological control and growth promoting agents in many economical valuable crops.

Moreover modern agriculture relies on high input of agrochemicals which cause major environmental problems in the world (Spiertz, 2010). Feeding an increasing human population and reducing the impacts on the environment urges for low input agricultural practices. Use of microorganisms in agriculture in order to promote the plant growth by circulating the nutrients in the soil is an alternative approach to reduce the need of chemical fertilizers as much as possible (Saharan and Nehra, 2011). In last few decades a wide variety of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia* have been reported to promote plant growth (Glick, 1995; Kloepper et al 1989; Okon and Labandera-Gonzalez, 1994). The direct promotion by PGPR entails either providing the plant with plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the soil. The indirect promotion of plant growth occurs when PGPR prevent deleterious effects of one or more phytopathogenic microorganisms. The exact mechanisms by which PGPR promote plant growth are not understood completely, but are thought to include the ability to produce IAA, HCN, Ammonia, Siderophores, solubilisation of phosphate (Joseph et al 2007; Manivannanet al 2012), and various enzyme like protease, catalase, amylase etc. Hence, our work was aimed to throw light on the alternative approaches like investigating indigenous PGPR for the presence of plant growth promoting traits and to select those PGPR which can be used for increasing the growth and yield of plants.

Materials and Methods

Sample collection

Soil samples were collected from rhizosphere of potato plants from four different sites, Turkadih, Lingiyadih, Sendri, River view colony of Bilaspur Chhattisgarh. The rhizosphere was dugout with intact root system. The samples were placed in plastic bags and stored at 4°C in refrigerator.

Isolation of PGPR from potato rhizosphere

Soil samples were collected from the rhizosphere of potato plants in different areas of Bilaspur, Chhattisgarh, India. Ten grams of collected rhizosphere soil were added in 100 ml of sterile triple de ionized water in flask separately. The flask was shaken for 25 min at 250 rpm on a rotary shaker. 1ml of suspension was added to 10 ml vial and shaken for 2 min. The suspension was serially diluted upto 12 fold. An aliquot (0.1 ml) of each dilution were spread on the plates of Luria-Bertany (LB) agar medium separately. Plates were incubated for 3 days at 28°C. The observations were recorded after 3 days of incubation. Each isolated colonies were transferred on LB slant for further investigation. The technique was perpetuated thrice and cultures were made single colony type.

Characterization of isolates

Morphological characteristics of the colony of each isolates were examined on LB agar plates, as method adopted by Manivannan et al., 2012. 3 days old culture of isolates bacterial colonies shape, size, elevation, surface, margin, colour, etc were recorded. The Gram's staining of the isolated strains was also carried out to find out the gram positive and gram negative strain.

Screening of PGP activites of isolated strains.

Determination of Indole Acetic Acid (IAA) production

Plant hormones can be natural or synthetic. There are several phytohormone groups and the best known is the auxin group. Diverse soil microorganisms including bacteria, fungi and algae are also capable of producing physiologically active quantities of auxins (IAA). IAA production was detected by the modified method as described by Fischer et al., 2007. Quantitative analysis of IAA was performed using the method of Loper and Scroth, 1986. The culture of 23 isolates were incubated in the LB broth at 28°C. The bacterial cells were removed from the culture medium by centrifugation at 8000 gm for 10 min. A 1ml of supernatant was mixed vigorously with 2ml of Salkowaski's reagent (4.5 gm of $FeCl_3$ per liter in 10.8 M H_2SO_4) and incubated at room temperature in the dark for 30 min. and observed the colour formation. Optical density was taken at 530 nm with the help of spectrophotometer using SL 191 UV VIS Double Beam Spectrophotometer (Elico).

Solubilisation of phosphate

The isolates were screened for phosphate solubilisation as per methodology adopted by Paul and Sinha, 2013. In this experiment colonies were transferred on Pikovskya's medium. The plates were incubated in an incubator at 28°C. The plates were then examined after 7 days of incubation and data were recorded. Visual detection of phosphate solubilizing ability of microorganisms is possible by plate screening methods that show clear zone around the microbial colonies in media containing insoluble mineral phosphates (tricalcium phosphate or hydroxyapatite) as sole P source.

Ammonia production

Important trait of PGPR is the production of ammonia that indirectly influences the plant growth. All the bacterial isolates were tested for the production of ammonia as described by Cappuccino and Sherman, 2010. 12 hrs old bacterial cultures were inoculated in peptone water (10 ml) in culture tube and incubated for 48-72 h at 36±2^{0}C. Development of brown to yellow colour after addition of Nesseler's reagent indicated positive test for ammonia, no colour change indicate negative test.

Siderophores production

Siderophore production was tested qualitatively using chrome azurol S medium (CAS-medium) method adopted by Manivannan et al., 2012. The culture of 23 isolates were streaked on the surface of CAS agar medium and incubated at room temperature for 1 to 3 days. Siderophore production was indicated by orange halos around the colonies after the incubation, and this test was done in three replications.

HCN production

Nutrient agar was amended with 4.4 g glycine/L and the culture was streaked on modified agar plate (Lorck, 1948). A Whatman filter paper (No. 1) soaked in 2% Sodium carbonate (in 0.5% picric acid) was placed at the top of the each isolated strain seperately. Plates were sealed with parafilm and incubated at 36±2°C for 4 days. Development of orange to red colour on the paper indicated HCN production.

Enzyme production test

***Catalase production test* (Aneja, 2003a)**

One loopful culture of each isolates were put on the slide and add few drop of H_2O_2 separately. The evolution of oxygen in the form of bubble indicate positive reaction (catalase poduction).

***Amylase production test* (Aneja, 2003b)**

For determination of amylase production, the starch agar medium (SAM) was used in the following composition peptone 5g, starch 20g, Agar 15g, distilled water 1 liter, and Beef extract 3g. All isolates strain were streaked on (SAM) medium separately and incubated at 37^{o}C for 48h. After 48h of incubation, flood 1% iodine solution for 5 minutes. The clear zone surround the bacterial colony indicate positive test for amylase production.

***Proteinase production test* (Aneja, 2003c)**

Protease producing ability of isolated bacteria was tested using Skim milk agar (SMA) medium in the following composition skimmed milk powder 28g, Casein enzymic hydrolysate 5g, yeast extract 2.5g, Dextrose 1g, Agar 15 g, and Distilled water 1litre. All of bacteria were streaked on (SMA) medium separately and incubated at 37^{o}C for 48 h. After 48 h of incubation, the colourless halos around the colony indicate positive test for protease production.

Results

Isolation of bacteria from potato rhizosphere soil

Twenty three bacterial isolates were isolated from rhizosphere soils of potato field from four different areas in Bilaspur. All the 4 sample representative bacteria were isolated, purified and maintained as pure cultures (**Table 1**).

Morphological characteristic of bacterial isolates

The morphological characteristics of PGPR isolates widely varied. Almost all the isolates produced round shaped and raised colonies having smooth shiny surface with smooth margin, only few produces filamentous, umbonate flat with rough and filamentous irregular margin was observed in the colonies of LB agar plate. Almost all the isolates were gram negative except few which are gram positive. They differed in colour but all were odourless. Diameter of the colonies of isolates varied from 0.2 to 0.7 mm (Table 2).

Growth promoting characteristic

Out of 5 isolates from Turkadih River view colony one produces HCN, four produces IAA and Ammonia, and none of them produces Siderophores and solubilises phosphorus. Out of 6 isolates from Lingiyadih one produces HCN, 5 produces Ammonia, 4 produces IAA, 5 produces ammonia, 3 solubilise phosphorus and none of them produces siderophores. Out of 4 isolates from Sendri, 3 produces HCN, and IAA, 2 produces siderophores and solubilises phosphorus and all produce ammonia. Out of 8 isolates from River view colony 4 produces HCN, 4 produces IAA, 1 produce siderophores, 7 produces ammonia, and none of them solubilises phosphorus (Table 3).

Enzyme production test

Observations of enzyme production test of rhizobacteria with consider to catalase amylase, and protease shows that out of 23 isolates 16 isolates were positive for catalase, 19 isolates were positive for Amylase, and 14 were positive for protease (Table 4).

Quantitative estimation of IAA Production

Isolates PGL4, PGS4, PGR3 and PGR4 were found to be good producers of IAA. On the contrary, PGT1, PGT3, PGT4, PGL1, PGL3, PGL5 was found to be a medium producer of IAA in comparison to the weak producer isolates PGT2, PGS1, PGS2, PGR6 and PGR7 (Table 5).

Discussion

Plant rhizosphere is known to be preferred ecological niche for soil microorganisms due to rich nutrient availability. The PGPR colonize roots of plant and enhance plant growth and development by a variety of mechanisms. The exact mechanism by which PGPR stimulate plant growth is not clearly known, although several mechanisms such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilisation and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion (Lavania et al 2006, Manivannan et al., 2012). There are many papers related to the screening and characterization of crop plants from Chhattisgarh particularly rice and sugarcane but in our knowledge few or none on Potato from Bilaspur, Chhattisgarh region. Very less information about screening and using PGPR with Potato crops are available. In present study, rhizobacteria were isolated from potato rhizosphere from different potato field of Bilaspur, Chhattisgarh, India. Isolated bacteria were screened for different plant growth promotion activities. Out of 23 isolates 5 bacterial isolates were positive for phosphate solubilisation. It has been reported that higher concentrations of phosphate-solubilising bacteria are commonly found in the rhizosphere soil as compared to nonrhizospheric soil (Reyes and Valduz, 2006). IAA is one of the most important phytohormone and function as important signal molecule in the regulation of plant development. It has been reported that IAA, applied at low concentrations, is known for its role in root development and cell division stimulation (Kishi et al., 2012). In our study most of the bacterial isolates were positive for IAA production. Another important trait of PGPR is the production of ammonia that indirectly influences the plant growth. Almost all the isolates were able to produce ammonia. Out of 23 bacterial strains 16 bacterial isolates in the present study were able to produce catalase. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical and chemical stress. HCN is a compound that is toxic to pathogen and its production by microbes are postulated to play an important role in the biological control of pathogens are reviewed by Babalola, 2010. In the present work, nine bacterial isolates were positive for HCN production, which acts as an inducer of plant resistance. Extracellular protease can also contribute to the ability of bacteria to suppress fungal diseases (Ghodsalavi et al., 2013). In the present study 14 out of 23 isolates were positive for protease. Amylase is the exoenzyme that hydrolyse starch in the present work's observation shows that 19 out of 23 was positive for amylase production. Multiple PGP activities among PGPR have been reported by some other workers (Husen, 2003; Joseph et al., 2007; Kumar et al., 2012; Sakthivel and Karthikeyan, 2012) while such findings on indigenous isolates of Bilaspur, Chhattisgarh India are less commonly explored. In the present study isolate PGS4 was found to be most efficient PGPR which solubilised insoluble iron, produced IAA, produced ammonia, HCN and also produce degrading enzyme viz, catalase and protease. Such type of study is necessary as it explore the potential of PGPR as inoculants or biofertilizers. Such kind of work are proved to be an effective alternate and ecofriendly approach to replace chemical fertilizers.

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Table1. Collection of samples for PGPR activities and isolation of various bacterial strain.

S.No.	Location of Potato rhizosphere soil	No. of isolates	Isolates Code
1	Turkadih	Five	PGT1,PGT2,PGT3,PGT4,PGT5
2	Lingiyadih	Six	PGL1,PGL2,PGL3,PGL4,PGL5,PGL6
3	Sendri	Four	PGS1,PGS2,PGS3,PGPS4
4	River view colony	Eight	PGR1,PGR2,PGR3,PGR4,PGR5,PGR6,PGR7,PGR8

Table 2. Morphological characteristics of 3-day-old colony of PGPR isolates.

S.No.	Isolates	Shape	Size of colony in mm	Elevation	Surface	Margine	Colour	Odour	Gram Staining
1	PGT1	Round	0.2-0.5	Raised	Smooth Shiny	Entire	White	Odourless	Gram -ve
2	PGT2	Round	0.4-0.6	Raised	Smooth Shiny	Entire	White	Odourless	Gram -ve
3	PGT3	Round	0.5-0.7	Raised	Smooth Shiny	Entire	Offwhite	Odourless	Gram –ve
4	PGT4	Round	0.2-0.5	Raised	Smooth Shiny	Entire	White	Odourless	Gram +ve
5	PGT5	Round	0.1-0.3	Raised	Smooth Shiny	Entire	White	Odourless	Gram –ve
6	PGL1	Round	0.2-0.4	Raised	Smooth Shiny	Entire	White	Odourless	Gram –ve
7	PGL2	Round	0.6-0.7	Raised	Smooth Shiny	Entire	Offwhite	Odourless	Gram –ve
8	PGL3	Round	0.2-0.5	Raised	Smooth Shiny	Entire	Yellow	Odourless	Gram +ve
9	PGL4	Filamentous	0.5-0.9	Raised	Rough	Filamentous	White	Odourless	Gram –ve
10	PGL5	Round	0.1-0.4	Raised	Smooth Shiny	Entire	White	Odourless	Gram –ve
11	PGL6	Round	0.3-0.4	Umbonate	Smooth Shiny	Entire	White	Odourless	Gram +ve
12	PGS1	Round	0.3-0.6	Raised	Smooth Shiny	Entire	White	Odourless	Gram –ve
13	PGS2	Filamentous	0.2-0.7	Raised	Rough	Filamentous	White	Odourless	Gram –ve
14	PGS3	Round	0.3-0.7	Convex	Smooth Shiny	Entire	Offwhite	Odourless	Gram –ve
15	PGS4	Filamentous	0.1-0.3	Flat	Smooth Shiny	Filamentous	White	Odourless	Gram –ve
16	PGR1	Round	0.2-0.3	Raised	Smooth Shiny	Entire	White	Odourless	Gram –ve
17	PGR2	Irregular	0.3-0.5	Raised	Rough	Irregular	Brownish	Odourless	Gram –ve
18	PGR3	Round	0.4-0.5	Raised	Smooth Shiny	Entire	White	Odourless	Gram –ve
19	PGR4	Round	0.1-0.3	Raised	Smooth Shiny	Entire	White	Odourless	Gram +ve
20	PGR5	Round	0.5-0.6	Raised	Smooth Shiny	Entire	White	Odourless	Gram –ve
21	PGR6	Round	0.2-0.5	Raised	Smooth Shiny	Entire	Yellow	Odourless	Gram –ve
22	PGR7	Round	0.4-0.3	Raised	Smooth Shiny	Entire	White	Odourless	Gram –ve
23	PGR8	Round	0.2-0.6	Raised	Smooth Shiny	Entire	Offwhite	Odourless	Gram –ve

Table 3. Plant growth promoting characteristics of Rhizobacterial isolates.

S.No	Bacteria strain	HCN	IAA	Siderophores	Ammonia	Phosphate solubilisation
Turkadih:						
1	PGT1	-	+	-	+	-
2	PGT2	-	+	-	+	-
3	PGT3	-	-	-	+	-
4	PGT4	+	+	-	-	-
5	PGT5	-	+	-	+	-

Lingiyadih:						
6	PGL1	-	+	-	+	-
7	PGL2	-	-	-	+	+
8	PGL3	-	+	-	+	-
9	PGL4	+	+	-	-	-
10	PGL5	-	+	-	+	+
11	PGL6	-	-	-	+	+
Sendri:						
12	PGS1	+	+	-	+	+
13	PGS2	-	+	-	+	+
14	PGS3	+	-	+	+	-
15	PGS4	+	+	+	+	-
River view colony:						
16	PGR1	+	-	-	+	-
17	PGR2	+	-	-	+	-
18	PGR3	-	+	-	+	-
19	PGR4	+	+	-	-	-
20	PGR5	-	-	-	+	-
21	PGR6	-	+	+	+	-
22	PGR7	-	+	-	+	-
23	PGR8	+	-	-	+	-

Table 4. Enzyme production test of isolated bacterial strains.

S.No	Bacteria strain	Catalase	Amylase	Protease
Turkadih:				
1	PGT1	-	-	-
2	PGT2	-	+	-
3	PGT3	-	+	+
4	PGT4	+	+	+
5	PGT5	-	+	+
Lingiyadih:				
6	PGL1	+	-	-
7	PGL2	+	+	-
8	PGL3	+	+	+
9	PGL4	-	+	+
10	PGL5	+	+	+
11	PGL6	+	+	-

Sendri:				
12	PGS1	-	+	+
13	PGS2	+	+	-
14	PGS3	+	+	-
15	PGS4	+	-	+
River view colony:				
16	PGR1	+	+	+
17	PGR2	+	-	+
18	PGR3	+	+	-
19	PGR4	-	+	-
20	PGR5	+	+	+
21	PGR6	+	+	+
22	PGR7	+	+	+
23	PGR8	+	+	+

Table 5. Bacterial isolates showing quantitative IAA production.

Bacterial isolates	IAA Production (µg/ml)
Control	0.00070±0.00030
PGT1	0.02873±0.00003
PGT2	0.00253±0.00027
PGT3	0.07060±0.00365
PGT4	0.01856±0.00012
PGT5	-
PGL1	0.31433±0.23583
PGL2	-
PGL3	0.33593±0.25203
PGL4	0.27713±0.00003
PGL5	0.03533±0.00003
PGL6	-
PGS1	0.00303±0.00013
PGS2	0.00166±0.00033
PGS3	-
PGS4	0.17523±0.00003
PGR1	-
PGR2	-
PGR3	0.13623±0.00003
PGR4	0.19933±0.00003
PGR5	-
PGR6	0.00690±0.00005
PGR7	0.00113±0.00003
PGR8	-

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