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ISOLATION AND CHARACTERIZATION OF BACTERIA FROM RHIZOSPHERIC SOILS OF *CURCUMA LONGA* FOR DIFFERENT PLANT GROWTH PROMOTION (PGPR) ACTIVITIES

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that can be found in the rhizosphere, in association with roots which can enhance the growth of plant directly or indirectly. A large number of bacteria including species of Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, Rhizobium and Serratia have reported to enhance plant growth. In the present study, six Crcuma Longa (Turmeric) soil samples were collected from different location of Tamil Nadu (Erode, Perundurai, Salem, Nammakkal, Alnganallur, Batlagundu, Gobi). A total thirty bacteria were isolated and in-vitro screening was done for different plant growth promotion activities i.e. phosphate solublization, IAA production, ammonia production, ACC deaminase activity, and catalase. In the present work twelve bacterial isolates were positive for phosphate solublization. IAA production was shown by almost all the bacterial isolates. Three isolates were positive for ammonia production. ACC deaminase activity was shown by nine isolates. Two isolates were positive for HCN production and all the isolates were found to be catalase positive. Seven isolates were showing maximum plant growth promotion activities and further identified on the basis of colony morphology, Gram staining and biochemical tests. These isolates were identified as Acinetobacter sp., Bacillus sp., Enterobacter sp., Micrococcus sp., and Pseudomonas sp. 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 Tamil Nadu.

KEYWORDS: Rhizobacteria, PGPR, Biofertilizers.

INTRODUCTION

The rhizosphere zone has been defined as the volume of soildirectly influenced by the presence of living plant roots or soil compartment influenced by the root. Rhizosphere supports large and active microbial population capable of exerting beneficial, neutraland detrimental effects on the plants. Rhizobacteria (root colonizing bacteria) that exert the beneficial effects on the growth of the host plant via direct or indirect mechanisms are termed as plant growth promoting rhizobacteria (PGPR). The plant-microbe interactions in the rhizosphere are responsible for increasing plant health and soil fertility.

PGPR strains use one or more direct or indirect mechanisms to enhance the growth and health of plants. These mechanisms can be active simultaneously or independently at different stages of plant growth. PGPR have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen, solublization of minerals such as phosphorus, production of siderophores, and synthesis of plant growth hormones i.e. Indole-3-acetic acid (IAA), gibberellic acid, cytokinins, and ethylene Indirect mechanisms involves the biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzymes, catalase and siderophore or through competition for nutrients and space can improve significantly plant health and promote growth, as evidenced by increases in seedling emergence, vigor, and yield. After N2 fixation, Phosphate (P) solubilization is very important plant growth promoting activity. A large proportion of soluble inorganic phosphate added to the soil is fixed as insoluble forms soon after the application and become unavailable to the plants. Several soil bacteria particularly belonging to genera Bacillus and Pseudomonas, posses the ability to change insoluble forms into soluble form by secreting organic acids as formic acid, acidic, propionic, lactic, glycolic, fumaric and succinic acid Biofertilizers such as microbial inoculants promote plant growth, productivity and increase the nutrient status of the host plant have internationally been accepted as an alternative source of chemical fertilizers. Significant increases in crop yields have been reported by applying PGPR microbial inoculants. So, keeping all this in view, the present study was carried out to isolate the various plant growth promoting strains from the rhizospheric soils of Tamil Nadu (Erode, Perundurai, Salem, Namakkal, Alanganallur, Gobi, Batlagundu).

MATERIALS AND METHODS

Soil sampling Soil samples were collected from the rhizosphere of Curcuma Longa plants growing at different (Erode, Perundurai, Salem, Namakkal, Alanganallur, Gobi Batlagundu) at Tamil Nadu in India. Intact root system was dug out and the rhizospheric soil samples were carefully taken in plastic bags and stored at 40C. Total of six soil samples were collected for the isolation of rhizosphere bacterial isolates.

Isolation of bacteria from rhizospheric soils

The P- solubilizers were isolated from the rhizospheric soil samples by serial dilution technique. Appropriate dilution was spread on Pikovskaya agar plate containing insoluble tricalcium phosphate. Plates were incubated at 30±0.1 0C for 24-48 h. Colonies showed halo zone were considered as P-solubilizer. The P-solubilizer. were purified by repeated streaking and stocked for further use.

Maintenance of isolates

All the isolates were maintained at 4°C in equal volumes of nutrient broth and 30% glycerol.

In vitro screening of isolates for different plant growth promoting activities. Phosphate solubilization

The isolates were screened for phosphate solubilization as per methodology described by Gupta S. et al.,[12] On modified Pikovskaya agar with insoluble tricalcium phosphate (TCP), a loop full of each culture was placed on the centre of agar plates and incubated at 30±0.1 °C for 5 days. The solubilization zone was determined by subtracting the diameter of bacterial colony from the diameter of total zone.

Quantitative estimation of phosphate

Quantitative estimation of inorganic phosphate solubilization was done as per methodology described by Nautiyal[13] and Jackson.[14] Bacterial isolates were grown in National Botanical Research Institute's Phosphate (NBRIP) broth containing 0.5% tricalcium phosphate (TCP). The flasks containing 50 ml medium was inoculated with 500 µl bacterial culture in triplicates and incubated at 30±0.1°C at 180 rpm for 5 days in Incubator Shaker. Simultaneously, the uninoculated control was also kept under similar conditions. The cultures were harvested by centrifugation at 10,000 rpm for 10 min. The phosphorus in supernatant was estimated by vanado-molybdate-yellow color method. To a 0.5 ml aliquot of the supernatant, 2.5 ml Barton's reagent was added and volume was made to 50 ml with de-

ionized water. The absorbance of the resultant color was read after 10 min at 430 nm in UV/Visible Spectrophotometer. The total soluble phosphorus was calculated from the regression equation of standard curve. The values of soluble phosphate liberated were expressed as µg ml-1 over control. The pH of culture supernatants were also measured using a pH Meter.

Detection of IAA Culture growth conditions

Fifty milliliter of Nutrient broth (NB) containing 0.1% DL-tryptophan was inoculated with 500 µl of 24 h old bacterial cultures and incubated in refrigerated incubator Shaker at 30±0.1°C and 180 rpm for 48 h in dark. The bacterial cultures were centrifuged at 10,000 rpm for 10 min at 4°C. Estimation of indole-3-acetic acid (IAA) in the supernatants was done using colorimetric assay.[15] Colorimetric estimation One millilitre of supernatant was mixed with 4 ml Salkowski reagent and absorbance of the resultant pink color was read after 30 min at 535 nm in UV/Visible Spectrophotometer. Appearance of pink color in test tubes indicated IAA production described by Gordon and Weber.[16] The IAA production was calculated from the regression equation of standard curve and the result was expressed as µg ml-1 over control.

Ammonia production

All the bacterial isolates were tested for the production of ammonia as described by Cappuccino and Sherman.[19] Overnight grown bacterial cultures were inoculated in 10 ml peptone broth and incubated at 30±0.1 °C for 48 h in Incubator Shaker. After incubation 0.5 ml of Nessler's reagent was added. The development of faint yellow to dark brown color indicated the production of ammonia.

Catalase activity: Catalase test was performed by taking a drop of 3% hydrogen peroxide was added to 48 hr old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The effervescence indicated catalase activity.

Biochemical identification of selected beneficial bacterial isolates

Seven isolates which showed maximum PGPR activity were further characterized by Gram staining and biochemical tests as per methodology described by Krieg and Holf.[21] The various tests performed were Oxidase, MR-VP, Indole, Citrate, Urease, Nitrate reduction and fermentation of various sugars. Recent Research in Science and Technology 2012, 4(1): 01-05 3.

RESULTS

Thirty bacterial isolates were isolated from the total of six rhizospheric soil samples from Curcuma Longa plant. All the isolates were designated as shown in Table no. 1. All isolates has shown significant PGPR activity. A total of thirty bacterial isolates were screened for phosphate solublization on modified PVK agar, of which twelve isolates showed the development of sharp phosphate solublization zones, ranging from 4 mm to 22mm. Other isolates showed the development of hazy zones. CLN5,CLA2 showed highest phosphate solublization i.e 22. mm and 15.3mm, respectively. Five isolates i.e. CLE4 (13.5) CLB3(18.5mm), CLS1(14.7) CLN5(21.0mm), CLA2(11.6mm), and CLB6(12.0mm) are produced zone greater than 10 mm, were further selected for quantification of phosphate solublization and further characterized for various PGPRs activities. In quantitative estimation, range of tri calcium phosphate solublization was between 15 to 60 µg/ml observed (Table 2). Most of the bacterial isolates produced plant growth promoting hormone i.e. IAA (Table 3). The range of IAA production was 0.2 to 213 µg/ml. Among all isolates, CLN5 prodced high IAA 212.61µg/ml whereas, CLN5, most efficient P-solubilizer was found to produced 212.61 µg/ml of IAA. Ammonia production is another important trait of PGPR that indirectly influence the plant growth. All the five efficient isolates were able to produced ammonia. Catalase activity was dedected in most of the bacterial isolates that may be potentially very advantageous.

Table 1. Description of the Bacterial isolates.

Sample Number	Location of Curcuma Longa Rhizosphere soil	No of isolates	Isolate codes
Sample 1	Erode (TN)	5	CLE1, CLE2, CLE3, CLE4, CLE5
Sample 2	Perundurai, Erode (TN)	4	CLP1, CLP2, CLP3, CLP4
Sample 3	Salem (TN)	5	CLS1, CLS2, CLS3, CLS4, CLS5
Sample 4	Namakkal (TN)	6	CLN1, CLN2, CLN3, CLN4, CLN5, CLN6
Sample 5	Alanganallur (TN)	4	CLA1, CLA2, CLA3, CLA4
Sample 6	Batlagundu (TN)	6	CLB1, CLB2, CLB3, CLB4, CLB5, CLB6

Table. 2. Bacterial isolates showing Quantitative P-solubilization.

Isolates codes	P-solubilization
CLE4	440.21
CLB3	363.27
CLS1	311.72
CLN5	560.31*
CLA2	482.46*
CLB6	369.25

Table 3. Bacterial isolates showing different plant growth promotion activities.

Isolates codes	P-solubilization	IAA production	Ammonia Production	ACC Deaminase activity	Catalase Activity
CLE4	+	123.48 µg/ml	+	+	+
CLB3	+	189.07 µg/ml	+	+	+
CLS1	+	102.56 µg/ml	+	+	+
CLN5	+	212.61 µg/ml	+	+	+
CLA2	+	160.24 µg/ml	+	+	+
CLB6	+	168.27 µg/ml	+	+	+

Table. 4. Biochemical characterization of bacterial isolates.

Biochemical Test	Cat	Oxi	I	MR	VP	C	Lac	Glu	Man	Su	U	Nit	Mo	Organism
CLE4	+	-	-	-	-	+	A	A	-	-	-	-	-	Acinetobacter sp.
CLB3	+	+	-	-	-	+	-	A	-	-	+	+	+	Pseudomonas sp.
CLS1	+	-	-	-	+	+	AG	AG	AG	AG	-	+	+	Enterobacter sp.
CLN5	+	-	-	-	-	+	-	A	-	A	-	+	-	Micrococcus sp.
CLA2	+	+	-	+	+	+	-	A	A	A	-	+	+	Bacillus sp.
CLB6	+	+	-	+	+	+	-	A	A	A	-	+	+	Rhizobium

Five isolates positively affected the germination of Pisum sativum and Zea mays seeds. Highest root elongation was recorded when seeds were pre-treated with CLN5 isolate. Bacterial isolates CLA2,CLE4, and CLB6 also showed the better ability to increase the length of root. These isolates were further identified by biochemical characterization. On the basis of biochemical characterization (Table 4) the most efficient P-solubilizer (CLN5) was identified as Pseudomonas sp.

CONCLUSION

PGPR colonize roots of plant and promote plant growth and development through 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 solubilization and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion.[22,23] There are many papers related to the advantages and screening of PGPR from crop plants particularly rice, maize and sugar cane but few on Curcuma Longa (turmeric). Little information about screening and using PGPR with Curcuma Longa (turmeric) is available. In present study, beneficial bacteria were isolated from Curcuma Longa rhizosphere. Isolated bacteria were screened for different plant growth promotion activities and characterized by biochemical tests. Five bacterial isolates were showed more than 10 mm zone of phosphate solubilization.

The isolate CLN5 (Pseudomonas sp.) showed highest phosphate solubilization zone (20 mm) in PVK agar. It has been reported that higher concentrations of phosphate-solubilizing bacteria are commonly found in the rhizosphere soil as compared to nonrhizospheric soil.[24] 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 production by PGPR can vary among different species and strains, and also influenced by culture conditions, growth stage and substrate availability.[25] Higher level of IAA production by Pseudomonas was recorded by other research workers.[26] 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. All the isolates were able to produce ammonia. All the 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. A number of studies suggest that PGPR enhances the growth, seed emergence, crop yield, and contribute to the protection of plants against certain pathogens and pests.[18,27,28,29,30] In current study, out of five isolates, isolate CLN5 (Pseudomonas sp.) significantly increased the root length of Pisum sativum and Zea mays seeds showed high ACC deaminase activity. Multiple PGP activities among PGPR have been reported by some other workers while such findings on indigenous isolates of India are less commonly explored.[32] In the present study isolate CLN5 (Pseudo sp) was found to be most Sufficient PGPR which solubilized insoluble phosphorus, produced IAA, produced ammonia, showed ACC deaminase activity and produced HCN and catalase. Such type of study is necessary as it advocates that use of PGPR as inoculants or biofertilizers is an efficient approach to replace chemical fertilizers.

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