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Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants

A. Suresh, P. Pallavi, P. Srinivas, V. Praveen Kumar, S. Jeevan Chandra and S. Ram Reddy*

Department of Microbiology, Kakatiya University, Warangal- 506009 (A.P), India.

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Fluorescent pseudomonads (FP), a major component of rhizobacteria, promote the plant growth through their multifarious activities. In the present investigations, 10 strains of fluorescent pseudomonads isolated from the rhizosphere soils of bajra (*Pennisetum glaucum*), jowar (*Sorghum vulgare*), rice (*Oryza sativa*) and maize (*Zea mays*) were screened for their plant growth promoting activity based on their ability to produce hydrogen cyanide (HCN), siderophores, proteases, indole acetic acid (IAA), broad spectrum antifungal activity against pathogenic fungi and phosphate solubilization. The results indicated that most of the isolates tested possess plant growth promoting traits. These isolates can be used as potential biofertilizers and also as biocontrol agents.

Key words: PGPR, indole acetic acid, hydrogen cyanide, protease, phosphate solubilization, antagonism.

INTRODUCTION

Many species and specific strains of bacteria residing in rhizosphere have been shown to possess plant growth promoting traits and hence they are collectively designated as plant growth promoting rhizobacteria (PGPR) (Gaskins et al., 1985). PGPR enhance plant productivity by a range of mechanisms. These beneficial effects of PGPR can be either direct or indirect. Direct promotion of growth by PGPR occurs when the rhizobacteria produce metabolites that promote plant growth such as auxins (Asghar et al., 2002), cytokinins (Arkhipova et al., 2005) and gibberellins (Gutierrez-Manero et al., 2001; Joo et al., 2004) as well as through the solubilization of phosphate minerals (Freitas et al., 1997). Indirect growth promotion occurs through the elimination of pathogens by the production of cyanide (Owen and Zlor, 2001) and siderophores (Pidello, 2003). PGPR beneficial effects have been exploited in many areas including biofertilizers, microbial rhizoremediation and biopesticides (Adesemoye et al., 2008).

FP, a major component of the plant growth promoting rhizobacteria are gram negative, motile, rod shaped bacteria and have a diverse phytobeneficial traits. Their plant growth promoting activities include production of HCN, siderophores, protease, antimicrobials, phosphate solubilizing enzymes (Chaiharn et al., 2008). However, these FP strains are reported to be crop specific and their beneficial effects may vary with the edaphic conditions (Abbas et al., 2009).

In the present investigations, we report the production of HCN, siderophores, protease, antimicrobials and phosphate solubilisation by 10 FP isolated from rhizosphere soils of four crop plants viz, maize, rice (SRI cultivar), bajra, jowar cultivated in different agro-edaphic conditions and non rhizosphere soils of Warangal (Dist) A.P. An attempt was made to isolate and screen the FP with an objective to develop them as bioinoculants, for selected crop plants

MATERIALS AND METHODS

Description of the study site

Soil samples were collected from rhizosphere soils of 4 crop plants viz, maize, rice (SRI cultivar), bajra, jowar and non-rhizosphere soils. The soils belonging to red loam or black loam of Paluvalpula, Mulugu and Regional Agriculture Research Center (RARC) Warangal.

Isolation and screening of fluorescent pseudomonads

Isolation of fluorescent pseudomonads was made from rhizosphere soils of 4 crop plants viz, maize, rice (system of rice intensification

*Corresponding author. E-mail: sanditi_ramreddy@yahoo.com.

(SRI) cultivar), bajra, jowar cultivated in different agro-edaphic conditions and non rhizosphere soils of Warangal (Dist) A.P, using King's B agar medium (King et al., 1954). After incubation at 3°C for 48 h the plates were exposed to UV light for few seconds and the colonies exhibiting the fluorescence were picked up and streaked on to the slants for maintenance and further characterization.

Assay for IAA production

The production of indole acetic acid (IAA) by selected 10 strains of fluorescent pseudomonads and the effect of L-tryptophan on IAA production was assayed by using Salkowski method (Glickman and Dessaux, 1995). The bacteria were inoculated in to the nutrient broth containing L-tryptophan concentrations of 0, 50, 100, 200, 300, 400 and 500 mg/l. After 48 h of growth, the bacterial culture was centrifuged and 1 ml of supernatant was mixed with 4 ml of Salkowski's reagent. The reaction mixture was incubated at room temperature for 20 mins and then light absorbance was measured immediately at 535 nm (Patten and Glick, 2002). The amount of IAA produced was calculated using the standard curve prepared with known concentration of IAA.

Assay for siderophore production

Production of siderophore was determined by Chromazurol Sulphonate (CAS) agar method (Alexander and Zuberer, 1991). Briefly, the bacterial inoculum was spotted into the center of a CAS agar plate. After incubation at 28°C for 5 days, siderophore production was assayed by the change in the colour of the medium from blue to orange.

Assay for protease production

Protease production was assayed using skim milk agar. Bacterial cells were spot inoculated and incubated for 2 days at 28°C. Proteolytic activities were identified by clear zone formation around the cell (Smibert and Krieg, 1994).

Assay for hydrogen cyanide production

Hydrogen cyanide production was assayed by the method suggested by Lorck (1948) and Castric (1977). For the production of HCN, bacteria were streaked into King's B agar plates supplemented with glycine. After this, petriplates were inverted and a piece of filter paper impregnated with 0.5% picric acid and 2% of sodium carbonate was placed on the lid. Petri plates were sealed with parafilm and incubated at 28°C for 96 h. Discoloration of the filter paper form orange to brown after incubation was considered as microbial production of cyanide.

Phosphate solubilization and estimation of soluble phosphate

To determine the solubilization of phosphate, the bacterial strains were streaked into Pikovskaya agar medium (Pikovskaya, 1948) and incubated at 28°C for 3 days. After 3 days, the colonies showing the clear zones around them were considered as positive. For the quantitative estimation of soluble phosphates, the bacterial strains were inoculated into 50 ml of pikovskaya (PVK) medium and incubated at 28°C with 160 rpm on rotary shaker. At different time intervals (1, 3, 5, 7 and 10 days) the culture broth samples were drawn and used for the estimation of soluble phosphate and then checking the pH of the culture medium. After centrifuging of the medium at 10000g for 15 min, 1 ml of the supernatant was taken and mixed with 3 ml of distilled water and 1 ml of molybdate-vanadate ammonium. After 20 min of incubation, the absorbance at 470 nm was measured. Phosphate solubility was determined using the standard curve of KH_2PO_4 (Jeon et al., 2003).

***In vitro* antifungal activity**

Antagonism on agar plates was studied by using modified method of Fokkema (1973). A mycelial disc (7 mm diameter) of the test fungus was aseptically transferred into the center of a fresh and dry malt extract agar (MEA) plate. Exponentially grown (24 h old) bacterial culture was streaked as a broad band approximately 40 mm away from the mycelia block. Control plates contained only fungal culture. The plates were incubated at 25°C and observed for 7 days. Fungal radial growth inhibition (a clear zone between the edges of fungal mycelia and bacterial colonies) was calculated (Fokkema, 1973) after 5 - 7 days of incubation.

Data analysis

The SPSS statistic program version 12.0 was performed for experiments involving calculations and the significance was evaluated by least significant differences (LSD) at $p < 0.05$.

Identification of the bacterial isolates

The 10 isolates were tentatively identified following Bergey's Manual of determinative bacteriology (Holt et al., 1994) and methods given by Cappuccino and Sherman (1993).

RESULTS AND DISCUSSION

All the 10 tested isolates of fluorescent pseudomonads were positive for the production of IAA, protease, siderophores and HCN (Table 1) . The rhizobacterial isolates showed clear visible haloes around their colonies on Pikovskaya agar medium after 3 days of incubation. Siderophores provide a competitive advantage to producer organism over fungal pathogens for the absorption of available iron (Jeffrey et al., 1999). The role of siderophores in the control of diseases has been well documented by Baker et al. (1986). Microbial production of HCN has been reported as an important antifungal trait to control root infecting fungi (Ramette et al., 2003).

IAA production by different strains ranged from 0 to 25.2 mg/ml with the highest amount recorded for the strain JPf7 (Table 2). However, when the media were supplemented with tryptophan, the IAA production increased substantially. The results of mean comparison related to different concentrations of L-tryptophan indicate that with increasing L-tryptophan concentrations, the amounts of auxin produced by bacterial strains has also increased which is in agreement with the results of Patten and Glick (2002). Ahamad et al. (2005) reported that 11 isolates of pseudomonads from different crop plants produced IAA with out tryptophan in the range 5.34 to 22.4 mg/ml. An increase of IAA production was observed in the presence of tryptophan. Similarly,

Karnwal (2009) also reported the varying amounts of IAA production by fluorescent pseudomonads.

Fluorescent pseudomonad strains are identified as potential phosphate solubilizers based on their ability to solubilize tricalcium phosphate [Ca_3 $(PO_4)_2$] by the formation of haloes on Pikovskaya agar medium. The present strains are capable of solubilizing Ca_3 $(PO_4)_2$ in the liquid medium (Table 3). The maximum amount of soluble phosphates was released by JPf2 (65.10 µg/ml) and the least by MPf1 (2.02 µg/ml) . These are significant at 0.05 level. The initial pH of the medium was 5.9. But due to the microbial production of organic acids the pH decreased to 4.9. Pandey and Palni (1998) reported *P. corrugata* as phosphate solubilizer. A significant decline in the pH of the culture medium suggests that the microbial production of organic acids (Illmer and Schinner, 1995). Most of the isolates showed decrease in pH of the medium with large variations, which is due to the variations in the release of organic acids (Nahas, 1996).

The fluorescent pseudomonad strains are reported to exhibit a broad spectrum of antifungal activity against phytopathogenic fungi. All the test isolates exhibited the antagonistic activity against four test fungi viz *Fusarium oxysporum, Curvularia lunata, Colletotricum falcatum, Macrophomina phaseolina in vitro*. Strains induced inhibition zones ranging from 0.1 to 2.7 cm towards phytopathogenic fungi. These are significant at 0.05 level. However, the antifungal activity varied both with the fluorescent pseudomonads strains as well as test fungus. MPf1, JPf2, RPf8, BPf30, MPf75, BPf1, JPf2, RPf3, MPf4 were significantly effective against all the phyto-pathogenic fungi. Maximum inhibitory effect was shown by JPf2 (Table 4). It was followed by JPf2 and MPf4. The lowest activity was shown by the strain MPf7. The antifungal activity of fluorescent pigment produced by fluorescent pseudomonads was observed by Cook et al. (1995) and Chythanya et al. (2002). Hassanein et al. (2009) reported that the antagonistic activity of some metabolites produced by *Pseudomonas aeruginosa* Sh8. Agarry and Osho (2005) reported *in vitro* and *in vivo* inhibition of *Aspergillus fumigatus* by *Pseudomonas fluorescence* using as a microbial antagonist. Shalini and Srivastava (2008) screened the antifungal activity of *P. fluorescence* against phytopathogenic fungi.

Thus, it is evident from the present studies that the fluorescent pseudomonads under investigation are capable of producing plant growth promoting substances and antifungal substances. Hence they are potential candidates for the development of bioinoculants for crop plants.

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