ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 6 (1): 49-52, 2007
ISSN 1682-296X

Screening for Beneficial Properties of Rhizobacteria Isolated From Sweetpotato Rhizosphere

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Abstract: This research highlighted in vitro study and was conducted to screen for the plant growth promoting characteristics of rhizobacterial strains isolated from sweetpotato rhizosphere. Fifteen rhizobacterial strains were screened for Indole Acetic Acid (IAA) production with and without addition of the L-Tryptophan (L-TRP), solubilization of phosphates and N_2-fixing activity. All of the isolates were able to produce IAA with higher production in the presence of L-TRP. Six of the isolates showed positive for phosphate-solubilizing activity and most of the isolates were able to grow on N-free media. The bacterial isolates showed differences in the growth promotion traits.

Key words: Sweetpotato rhizosphere, PGPR, indole acetic acid, phosphate solubilization

INTRODUCTION

Beneficial effects of PGPR on plant growth have been attributed to mechanisms such as production of phytohormones, solubilization of phosphates and asymbiotic nitrogen fixation. Several species of PGPR are able to produce phytohormones such as auxin, cytokinin, gibberellins and ethylene like substances (Frankenberger and Arshad, 1995). One of the phytohormones produced by soil microorganisms Is Indole-3 Acetic Acid (IAA) which is an important hormone for plant growth and development. Different bacterial species were also able to solubilize complex inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate (Goldstein, 1986). Soil inoculation with phosphate solubilizing bacteria was known to improve solubilization of fixed soil phosphorus and applied phosphates resulting in higher crop yields (Abd-Alla, 1994). Nitrogen fixation was carried out by associative and free-living microorganisms in the rhizosphere of plants which has been recognized to play an important role in nitrogen nutrition of plants (Boddey *et al*., 1996). Colonization of bacteria with the beneficial traits promoted and stimulation of plant mechanisms (Sindhu *et al*., 1999). Exploitation of this type of bacteria to improve crop of production has become important in sustainable agriculture. Therefore, this study was conducted to screen for the activities of IAA production, phosphate solubilization and nitrogen production by rhizobacterial strains isolated sweetpotato rhizosphere.

MATERIALS AND METHODS

Fifteen isolates obtained from sweetpotato rhizosphere and screened for the ability to produce IAA, solubilization of phosphate and nitrogen fixing activity.

IAA production: A modified colorimetric method was used for determination of IAA (Asghar *et al*., 2000). The selected rhizobacteria isolates were grown in king's B broth (25 mL) with and without (5 mL) L-TRP (0.5%) solution and shaked at 28±1°C for 24 h and then centrifuged for 10 min. One milliliter culture supernatant was placed in a test-tube and mixed with 2 mL Salkowsky reagent (Gordon and Weber, 1950). After 20-25 min, the colour absorbance were read using spectrophotometer at 535 nm. Pure IAA was used for preparing the standards of 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45 μg mL^{-1}.

Solubilization of phosphate: The ability of isolates to solubilize phosphate was assessed using Potato-Dextrose Yeast Extract Agar (PDYA) containing freshly precipitate of $CaHPO_4$. Each bacterial culture were spot inoculated in the centre of a PDYA-CaP plate and incubated at 30°C for 10 days. Phosphate solubilization was assessed by measuring the clearzone diameter (Freitas *et al*., 1997).

Total nitrogen accumulation: The capability of isolates to fix nitrogen was determined by their ability to grow in nitrogen free broth media. Bacterial isolates were cultured one day in nitrogen free broth media (Xie *et al*., 2003) with

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shaking for 24 h at 28±2°C. The content of the culture filtrate was subjected to kjeldahl digestion and total N was determined (Bremner and Mulvaney, 1982).

RESULTS

IAA production: Results showed that the fifteen rhizobacterial isolates were able to produce IAA The concentration of IAA produced by the rhizobacterial isolates ranged between 3.84 to 13.33 μg mL^{-1} (Fig. 1).

Higher IAA production was observed in the presence of precursor L-TRP. The concentration of IAA produced ranged between 4.97 to 46.66 μg mL^{-1}. There was difference in the concentration of IAA produced between the isolates. However, isolate UPMSP9 seemed to produce high amount of IAA while isolate UPMSP1 produced very low amount of IAA in culture medium (Fig. 2).

Solubilization of phosphate: Six out of fifteen rhizobacterial isolates were able to form clear zone on PDYA, an indication of Calcium phosphate solubilization. The six isolates were UPMSP10, UPMSP3, UPMSP9, UPMSP18, UPMSP6 and UPMSP20 seemed to the phosphate solubilizing rhizobacteria. Highest clear zone was observed in UPMSP10 and the smallest clear zone was formed by UPMSP20 (Table 1).

Total nitrogen accumulation: All rhizobacterial isolates were able to grow in N free broth and increased

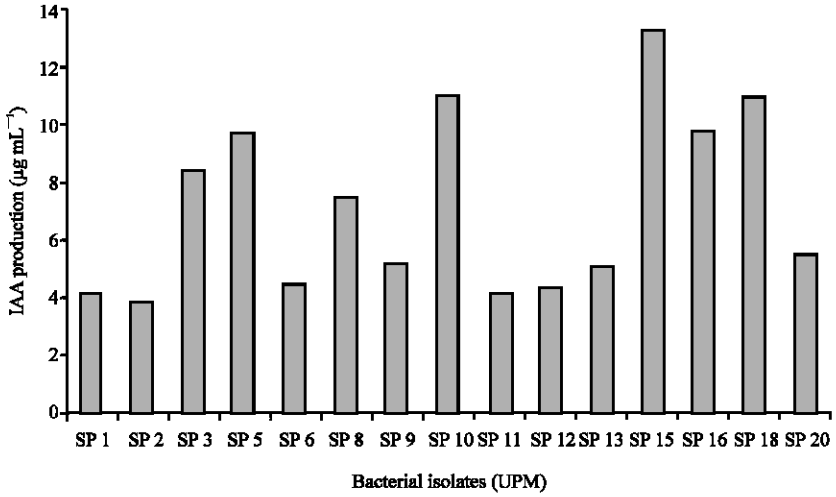

Fig. 1: Production of IAA by the Rhizobacterial isolates (without L-TRP)

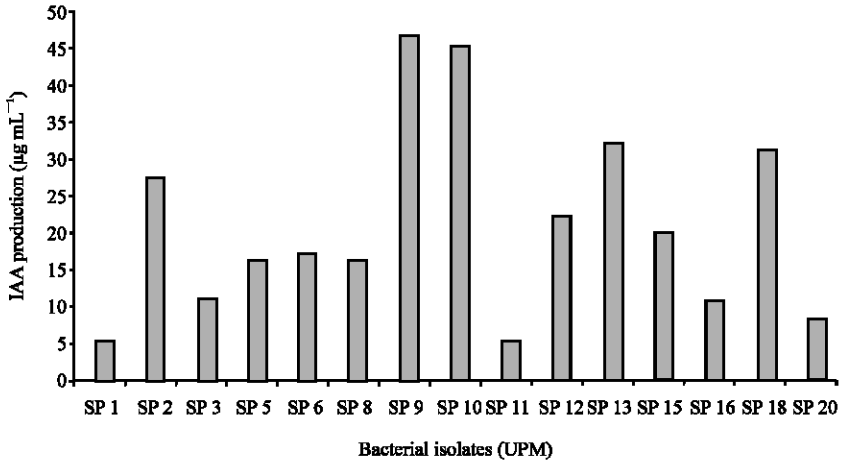

Fig. 2: Production of IAA by the Rhizobacterial isolates (with L-TRP)

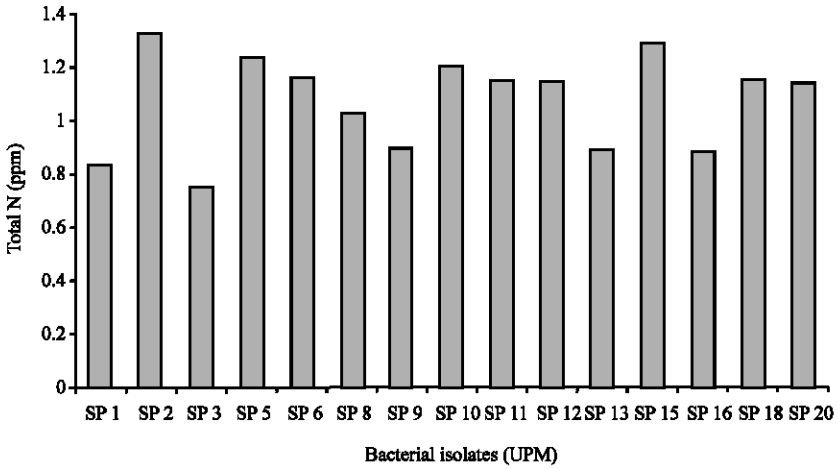

Fig. 3: Total Nitrogen accumulation by the Rhizobacterial isolates

Table 1: P solubilizing activity of Rhizobacterial isolates

Isolates	P solubilization	Diameter of clearzone (mm)
UPMSP 1	-	-
UPMSP 2	-	-
UPMSP 3	+	18.5
UPMSP 5	-	-
UPMSP 6	+	10.7
UPMSP 8	-	-
UPMSP 9	+	12.3
UPMSP 10	+	20.3
UPMSP 11	-	-
UPMSP 12	-	-
UPMSP 13	-	-
UPMSP 15	-	-
UPMSP 16	-	-
UPMSP 18	+	12.0
UPMSP 20	+	8.6

the total N in the growth culture (Fig. 3). The amount of N produced differed between isolates. Isolates UPMSP 2, UPMSP 5 and UPMSP 15 produced higher amount of N (1.24 to 1.32 ppm) while isolates UPMSP 3 and UPMSP 1 produced very low amount of N (0.74 to 0.83 ppm).

DISCUSSION

The present study, clearly revealed that all isolates tested in this study had the ability to produce IAA. Consequently they were considered as IAA producing rhizobacteria. The concentration of IAA produced varies with strains. The highest IAA was 13.33 μg mL^{-1} byUPMSP 15 isolate. Studies by Leinho and Vacek (1994) reported IAA production by *Pseudomonas* and *Acinotobacter* isolated from wheat and rye rhizosphere ranging from 0.01 to 3.98 mg L^{-1}. Hundred bacterial isolates produced IAA in the absence of precursor L-TRP and the highest concentration of IAA produced by one of the isolate was 11.40 μg mL^{-1} (Asghar *et al*., 2000). Bacterial isolates obtained from the rhizosphere of various plants, which help to produced 86% IAA in pure culture (Barea *et al*., 1976).

Most of the isolates produced higher IAA in the presence of the precursor, L-TRP. The highest IAA was 46.66 μg mL^{-1} by UPMSP9. Three of the isolates UPMSP 9, UPMSP 2 and UPMSP13 showed 9, 7 and 6-fold increases, respectively, in IAA when grown in media with L-TRP. This shows that some strains are dependent on L-TRP precursor and probably synthesized IAA through TRP pathways. L-TRP (an auxin precursor) addition to the media increased the auxin production by several fold. The highest concentration of IAA produced by one of the isolate was 115.50 μg mL^{-1} the addition of L-TRP to the medium (Asghar *et al*., 2000). Under the natural condition, plant roots excrete organic compounds including L-TRP which can then be utilized by the rhizobacteria for IAA biosynthesis.

Six out of fifteen rhizobacterial isolates showed the ability to solubilize calcium phosphate on the plate assay consequently, they were considered as phosphate-solubilizing rhizobacteria. Soil inoculation with phosphate-solubilizing bacteria has been shown to improve solubilization of fixed soil P and applied phosphates resulting higher crop yields (Freitas *et al*., 1997; Nautiyal, 1999). Several phosphate-solubilizing rhizobacteria could also promote plant growth. It render phosphate into solution more than they need for their metabolism and the surplus can be absorbed by plant (Kloepper *et al*., 1989; Rodriguez and Fraga, 1999).

Most of the isolates were able to grow on N-free broth and increased the total N in the growth culture. This presumptive test indicates the N_2 fixing activity of the isolates. More direct methods need to be conducted to confirm the activity. Nitrogen fixation carried out by associative and free-living microorganisms in the rhizosphere of plants has been recognized to play an important role in nitrogen nutrition of plants (Boddey *et al*., 1996; Zubber, 1998).

In conclusion, the study indicated that about two isolates UPMSP9 and UPMSP10 possesses the ability to produce high IAA with L-TRP, solubilize phosphate and produce N in culture medium. These isolates have the potential to be used as plant biofertilizer and bioenhancer for plant growth improvement

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