PRODUCTION OF INDOLE ACETIC ACID BY FLUORESCENT *PSEUDOMONAS* IN THE PRESENCE OF L-TRYPTOPHAN AND RICE ROOT EXUDATES

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SUMMARY

A total of 30 fluorescent *Pseudomonas* isolates (15 *P. fluorescens* and 15 *P. aeruginosa*) were obtained from different plant rhizospheres and were characterized on the basis of biochemical tests and plant growth-promoting activities. *Pseudomonas fluorescens* AK1 and *Pseudomonas aeruginosa* AK2 showed the best plant growth-promoting activity. These isolates were tested for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan at 50, 100, 200 and 500 µg/ml. For both strains, indole production increased with increases in tryptophan concentration (0.5, 1.2, 4.3 and 9.3 µg/ml; and 0.2, 0.7, 3.8, and 8.3 µg/ml, respectively). *P. aeruginosa* AK2 was less effective in production of indole acetic acid than *P. fluorescens* AK1. Inoculation of rice seeds with *P. fluorescens* AK1 and *P. aeruginosa* AK2 showed a good level (2.30 pmol/ml and 2.1 pmol/ml) of indole acetic acid compared to uninoculated seeds (1.6 pmol/ml).

Key words: Plant growth promoting rhizobacteria, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, biochemical test, PGPR activity.

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, which enhance the growth of the plant either directly or indirectly (Kloepper *et al*., 1980; Glick, 1995). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production, production of phytohormones such as auxins, cytokinins and gibberellins, and lowering of ethylene concentration (Kloepper *et al*., 1989; Glick, 1995; Glick *et al*., 1999). Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* as well as *Alcaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter diazotrophicus* and *radyrhizobium japonicum* have been shown to produce auxins which help in stimulating plant growth (Patten and Glick, 1996).

Azotobacter paspali significantly increased the dry weight of leaves and roots of several plant species following root treatment by production of indole acetic acid (IAA) (Barea and Brown, 1974). It has been reported that production of IAA and ILA (indole-3-lactic acid) increased with increasing concentrations of tryptophan (1-100 µg/ml) by *Azospirillum brasilense* Sp13t and SR2, respectively (Tien *et al*., 1979).

In wheat, *A. brazilense* inoculation of wheat seedlings increased the number and length of lateral roots (Barbieri *et al*., 1986). Inoculation of canola seeds with *Pseudomonas putida* GR12-2, which produces low levels of IAA, resulted in 2 to 3 fold increase in the length of seedling roots (Glick *et al*., 1986; Caron *et al*., 1995).

It is assumed that plant growth regulators produced by *Pseudomonas* species could also influence plant growth. To assess auxin production and its influence on the plant, some 30 strains (both *P. fluorescens* and *P. aeruginosa*) were screened for their natural ability to produce IAA in the presence of varying amounts of L-tryptophan and rice root exudates.

MATERIALS AND METHODS

Isolation and biochemical characterization of *Pseudomonas* strains. Fluorescent *Pseudomonas* species were isolated from soils collected from Haridwar, Nanital, Haldwani, Srinagar and Dehradun regions of Uttarakhand state (India). The serial dilution agar plate method was used to isolate *Pseudomonas* species on nutrient agar medium or King's B medium. Pigmentation and biochemical reactions were determined as described in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). All the 15 isolates of *P. fluorescens* and 15 isolates of *P. aeruginosa* were biochemically characterized for Gram staining, starch hydrolysis, H_2S production, citrate utilization, oxidation reaction, casein hydrolysis, 3-ketolactose production, urease production, catalase test, lipolysis activity, indole production, pigment production, lipolysis activity, HCN production and gelatin liquefaction.

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Screening of bacterial isolates for indoles and indole acetic acid (IAA) production. Production of indoles by *P. fluorescens* AK1 and *P. aeruginosa* AK2 was assayed as described by Patten and Glick (1996). Bacterial isolates were propagated in DF salts minimal medium with L-tryptophan (0, 50, 100, 200 and 500 µg/ml) and incubated at 28±2°C for 48 h. Bacterial cells were removed by centrifugation at 4,000 rpm for 20 min at 4ºC. One ml of the supernatant was mixed with 4 ml of Salkowski's reagent in the ratio of 1:4 and incubated at room temperature for 20 min. Development of a pink colour indicated indoles. The absorbance of supernatant mixture (supernatant+ Salkowski's reagent) for indole production was measured at 535 nm The quantity of indoles was determined by comparison with a standard curve using an IAA standard graph. Indole-3-acetic acid production by *P. fluorescens* AK1 and *P. aeruginosa* AK2 was estimated by ELISA. Supernatants from cultures grown in DF salts minimal medium amended with 0, 100, 200 and 500 µg/ml L-tryptophan were assayed. Three-milliliter aliquots of supernatant were methylated by adding four to five drops of 2.0 M trimethylsilyl diazomethane in diethyl ether, then samples were vortexed at high speed for 1 min and placed in a fume hood to evaporate excess ether.

The colour produced on addition of substrate is inversely proportional to the amount of phytohormone in the sample. The intensity of the colour was read at 405 nm using an ELISA plate reader and related to phytohormone concentrations by means of a standard curve.

Effect of rice roots on IAA production in growth chamber studies. In a growth pouch study, rice seeds (*Oryza sativa*, cv. Basmati type-3) were surface sterilized in 95% ethanol (v/v) for 10 to 20 sec, then soaked in 20% bleach (v/v) for 10 min. The seeds were washed with sterile distilled water 5-7 times to remove excess bleach and were air dried in a laminar flow hood for 24 h. Seed growth pouches sterilized at 121ºC for 15 to 20 min were filled with 10 ml of sterile half-strength N-free Hoagland's nutrient solution. Bacterial cells grown in half strength tryptic soy broth (TSB) were diluted 100-fold. Surface-sterilized seeds were soaked in 10 ml of bacterial suspension for 10-15 min with gentle agitation. The growth pouches were filled with 25 ml of Hoagland's N-free nutrient solution and seeds treated with bacterial strains were transferred aseptically to growth pouches (3 seeds per pouch and 3 pouches per treatment). Seeds treated with 0.1 M $MgSO_4$ served as controls. The growth pouches were incubated in a growth cabinet for 72 h with gentle shaking at 100 rpm to create aerobic conditions. Supernatants from growth pouches (10 ml) were obtained by centrifugation at 4,000 rpm for 20 min at 4°C and filtration using 0.22 µm membrane filters. The filtrate was used to detect and quantify the concentration of IAA production by *P. fluorescens* AK1 and *P. aeruginosa* AK2 by ELISA. Aliquots of filtrates (3 ml) were methylated by adding four to five drops of 2.0 M trimethylsilyl diazo-methane in diethyl ether, then samples were vortexed at high speed for 1 min and placed in a fume hood to evaporate excess ether.

IAA was assayed using ELISA kits (Phytodetek, Agdia, USA). Stock solutions of the IAA (10 µmole/ml) were prepared in absolute methanol. Standard concentrations of 78-2500 pmoles/ml (IAA) were used. One hundred microlitre of standard or the sample were used for each assay.

RESULTS AND DISCUSSION

All of the 30 isolates of fluorescent *Pseudomonas* recovered from soils of different regions of Uttarakhand were gram-negative, citrate-positive, oxidase-positive, catalase-positive, indole-positive, produced fluorescence under UV light, were able to hydrolyze starch and casein, produced siderophores and were able to use glucose, mannitol, fructose, arabinose, trehalose, glycerol, xylose and starch as carbon source. These isolates were screened for their ability to produce IAA and other PGPR activity. *P. fluorescens* AK1 and *P. aeruginosa* AK2 showed better PGPR activity than other isolates. In the absence of L-tryptophan, *P. aeruginosa* AK2 produced significantly higher amounts of indole (0.8 µg/.ml) than *P. fluorescens* AK1 strain (0.2 µg/ml). In the presence of 50 µg/ml of L-tryptophan *P. fluorescens* AK1 produced a significantly higher concentration of indole (1.4 µg/ml) than *P.aeruginosa* AK2 (0.9 µg/ml). When 100 µg/ml L-tryptophan was added to the medium *P. fluorescens* AK1 and *P. aeruginosa* AK2 produced three (4.0 µg/ml) and four (3.9 µg/ml) times, respectively the concentration of indole produced at the 50 µg/ml L-tryptophan concentration. A significant increase in indole production was recorded in the presence of 200 µg/ml and 500 µg/ml L-tryptophan, i.e. 4.3 µg/ml and 9.3 µg/ml, respectively, for *P. fluorescens* AK1. The same increment in indole production was noted with *P. aeruginosa* AK2 4.1 µg/ml and 8.2 µg/ml at 200 µg/ml and 500 µg/ml of L-tryptophan concentration, respectively.

Varying levels of IAA were recorded with different concentrations of tryptophan, i.e. 0, 100, 200 and 500 µg/ml. The concentration of IAA in *P. fluorescens* AK1 isolates without tryptophan was 3.1 pmol/ml. A significant increase in the production of IAA was recorded in the presence of 100, 200 and 500 µg/ml of tryptophan, i.e. 3.8, 5.2 and 6.9 pmol/ml, respectively. Similarly, *P. aeruginosa* AK2 was able to produce IAA without tryptophan in a concentration of 3.3 pmol/ml. A further increase in IAA production (3.9 pmol/ml, 4.0 pmol/ml and 4.2 pmol/ml) was observed in the presence of different concentrations of L-tryptophan (100, 200 and 500

µg/ml). Both isolates produced IAA as observed by others (Narumiya *et al.*, 1979; Bano and Musarrat, 2003). The concentrations of IAA secreted with rice root exudates in growth chamber studies with *P. fluorescens* AK1 and *P. aeruginosa* AK2 were significantly higher (2.3 pmol/ml and 2.1 pmol/ml) than that of the control (1.6 pmol/ml).

These results proved that plant growth regulators produced by *Pseudomonas* species could also play a critical role in plant growth promotion.

REFERENCES

Bano N., Musarrat J., 2003. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. *Current Microbiology* **46**: 324-328.

Barbieri P., Zanelli T., Galli E., Zanetti G., 1986. Wheat inoculation with *Azospirillum brazilance* Sp6 and some mutants altered in nitrogen fixation and indole-3-acetic acid production. *FEMS Microbiology Letters* **36**: 87-90.

Barea J.M., Brown M.E., 1974. Effects on plant growth by *Azotobacter paspalirelated* to synthesis of plant growth regulating substances. *Journal of Applied Bacteriology* **37**: 583-593.

Caron M., Patten C.L., Ghosh S., 1995. Effects of plant growth promoting rhizobacteria *Pseudomonas putida* GR-122 on the physiology of canola roots. *Proceedings of the Plant Growth Regulation Society of America* **7**:18-20.

Dunne C., Crowley J.J., Loccoz Y.M., Dowling D.N., de Bruijn F., O'Gara F., 1993. Biological control of Pythium *ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. *Microbiology* **143**: 3921-3931.

Glick B.R., 1995. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* **41**: 109-117.

Glick B.R., Brooks H.E., Pasternak J.J., 1986. Physiological effects of plasmid DNA transformation of *Azotobacter vinelendi*. *Canadian Journal of Microbiology* **32**: 145-148.

Glick B.R., Patten C.L., Holguin G., Penrose D.M., 1999. Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria. Imperial College Press, London, UK.

Holt J.G., Krieg N.R., Sneath P.H.A., Staley J.T., Williams S.T., 1994. Bergey's Manual of Determinative Bacteriology, 9th Ed. Williams & Wilkins, Baltimore, MS, USA.

Kampert M., Strzelczyk E., Pokojska A., 1975. Production of auxins by bacteria isolated from the roots of pine seedlings (*Pinus silvestris* L.) and from soil. *Acta Microbiologica Polonica* **7**: 135-143.

Kloepper J.W., Leong J., Teintze M., Schroth M.N., 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature* **286**: 885-886.

Kloepper J.W., Lifshitz R., Zablotowicz R.M., 1989. Free-living bacterial inocula for enhancing crop productivity. *Trends in Biotechnology* **7**: 39-44.

Kloepper J.W., Hume D.J., Scher F.M., Singleton C., Tipping B., Laliberté M., Frauley K., Kutchaw T., Simonson C., Lifshitz R., Zaleska I., Lee L., 1988. Plant growth-promoting rhizobacteria on canola (rapeseed). *Plant Disease* **72**: 42-45.

Liu L., Kloepper J.W., Tuzun S., 1995. Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria. *Phytopathology* **85**: 843-847.

Loper J.E., Schroth M.N., 1986. Influence of bacterial sources of indole-2-acetic acid on root elongation of sugar beet. *Phytopathology* **76**: 386-389.

Narumiya S., Takai K., Tokuyama T., Noda Y., Ushiro H., Hayaishi O., 1979. A new metabolic pathway of tryptophan initiated by tryptophan side chain oxidase. *Journal of Biological Chemistry* **254**: 7007-7015.

Patten C., Glick B.R., 1996. Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology* **42**: 207-220.

Tien T.M., Gaskins M.H., Hubbell D.H., 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology* **37**: 1016-1024.

Weller D.M., Cook R.J., 1986. Increased growth of wheat by seed treatments with fluorescent pseudomonads and implications of *Pythium* control. *Canadian Journal of Plant Pathology* **8**: 328-334.

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