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PRODUCTION OF INDOLE ACETIC ACID (IAA) BY RHIZOBACTERIA OF PADDY (*ORYZA SATIVA* L.)

Biotechnology

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ABSTRACT

BACKGROUND: The soil bacteria that colonize the roots and enhance plant growth are called plant growth promoting rhizobacteria (PGPR). Plant growth promoting substances produced by rhizobacteria include Indole acetic acid (IAA), Siderophores and enzymes for solubilization of inorganic phosphates. Indole-3-acetic acid is an auxin (phytohormone) responsible for division, extension and differentiation of plant cells and tissues.

AIM: To screen the capacity of IAA produced by rhizobacteria.

OBJECTIVE: To study the IAA producing ability of rhizobacteria

METHODS: Rhizosphere bacteria were isolated from rhizosphere zone of *Oryza sativa* by serial dilution technique. Production of IAA by the bacterial isolates was screened using Jeon's medium. Pure culture of each of the rhizobacterial isolates was used to screen IAA production. 2 mL of supernatant of each of the bacterial isolates was mixed with 2 mL of Salkowski's reagent and incubated at 25 ±2^{0}C for 30 minutes in dark. The development of pink colour indicated the presence of IAA, and the concentration of IAA produced by rhizobacterial isolates was measured spectrophotometrically at 530 nm and quantified in IAA standard curve in terms of mg of IAA/L.

RESULTS: All the rhizobacterial isolates showed positive IAA production capabilities at different concentrations in the presence of L-Tryptophan. *Micrococcus luteus, Azotobacter beijerinckii, Pseudomonas fluorescens, Pseudomonas aeruginosa, Agrobacterium fabrum, Flavobacterium anhuiense, Burkholderia* sp. and *Rhizobium* sp. produced highest amount of IAA, in the concentration of 10.5 ±0.03, 11.5 ±0.11, 10.6 ±0.07, 10.5 ±0.08, 11.7 ±0.12, 11.6 ±0.09, 11.8 ±0.13 and 11.8 ±0.06 mg/L of IAA respectively.

CONCLUSIONS: The present PGPR Rhizobacterial isolates can be used as an inoculants biofertilizers in rice or other cereal growing fields to replace chemical fertilizers.

KEYWORDS

PGPR, *Oryza sativa*, Indole acetic acid, Rhizosphere

INTRODUCTION

The soil bacteria that colonize the roots and enhance plant growth are called plant growth promoting rhizobacteria[1]. In the rhizosphere about 2-5% of bacteria are PGPR[2]. PGPR can be classified into three types viz. extracellular PGPR found in the rhizosphere (ePGPR), on the rhizoplane (sPGPR), in the intercellular spaces of root cortex (iPGPR) existing inside the root tissue[3].

Rhizobacteria produce plant growth promoting substances which enhance plant growth directly and indirectly[4-8]. Plant growth promoting substances include Indole acetic acid (IAA), Gibberellins, Siderophores and enzymes for solubilization of inorganic phosphates. Indole-3-acetic acid is an auxin (phytohormone) responsible for division, extension and differentiation of plant cells and tissues[5,9-11]. Auxin (Indole-3-Acetic acid) is essential for growth of plants, and is produced by many rhizobacteria viz. *Streptomyces, Methylobacteria, Cyanobacteria* and *Archaea*. Gibberellins are tetracyclic diterpenoid acids involved in many physiological processes of plants including seed germination, seedling emergence, stem and leaf growth, flower induction and fruit growth. Many rhizobacteria viz. species of *Azospirillum*, *Rhizobium*, *Azotobacter*, *Arthrobacter*, *Pseudomonas*, *Bacillus*, *Acenetobacter*, *Flavobacterium*, *Micrococcus*, *Agrobacterium*, *Clostridiuum*, *Burkholderia*, and *Xanthomonas* are known to synthesize gibberellins. The present work is aimed to investigate the production of IAA by the rhizobacteria isolated from paddy fields of Pandaul (Madhubani).

MATERIALS AND METHODS

The soil sample of paddy growing fields was analysed by serial dilution (10^{-1} to 10^{-5}) in a sterile physiological saline. 0.1 mL of the various dilutions was poured onto freshly prepared nutrient agar medium and spread evenly with a sterile bent glass and incubated at 28±2^{0}C for 48 hours.

The inoculum was prepared by transferring the loopful of each of the bacterial isolates into 200 mL conical flasks containing 30 mL of sterile tryptic soy broth separately. The cultures were incubated on a rotary shaker at 150 rpm at 25 ±2^{0}C for 24 hours. The cultures were then centrifuged at 3000 rpm for 15 minutes, washed in physiological saline and re-suspended in sterile distilled water. The optical density (OD) of the bacterial suspension was adjusted to 0.6 at 600 nm.

Production of IAA by the bacterial isolates was screened using Jeon's medium as suggested by Jeons *et al*[6]. Pure culture of each of the rhizobacterial isolates was inoculated into 5 mL of Jeon's medium separately in 20 mL tubes. The tubes were incubated at 25 ±2^{0}C for 72 hours. The culture was then centrifuged at 3000 rpm for 10 minutes and the supernatant was used for screening of IAA production. 2 mL of supernatant of each of the bacterial isolates was mixed with 2 mL of Salkowski's reagent (2 % $FeCl_2$ in 35 % perchloric acid) and incubated at 25 ±2^{0}C for 30 minutes in dark. The development of pink colour indicated the presence of IAA, and the concentration of IAA produced by rhizobacterial isolates was measured spectrophotometrically at 530 nm and quantified in IAA standard curve in terms of mg of IAA/L.

In the present investigation, the rhizobacterial isolates of paddy growing fields viz., *Bacillus subtilis, B. megaterium, B. pumilus, B. polymyxa, Lactobacillus acidophillus, L. plantarum, L. brevis, Micrococcus luteus, Azotobacter beijerinckii, Pseudomonas putida, P. fluorescens, P. aeruginosa, Streptococcus salivaris, Staphylococcus saprophyticus, Erwinia amylovora, Enterobacter hormechei, Agrobacterium fabrum, Flavobacterium. Anhuiense, Acinetobacter soli, Burkholderia sp., Rhizobium sp. and Serratia sp., Nitrosomonas sp., Nitrosococcus sp., Nitrobacter sp., Nitrospira sp. and Nitrosovibrio sp.* were screen for the capacity of production of IAA. The results obtained have been presented in Table-1 and Figure-1.

Table-1: Production of Indole Acetic Acid (IAA) by rhizobacterial isolates

Rhizobacterial isolates	IAA mg/L
Bacillus subtilis	6.8 ±0.06
Bacillus megaterium	6.6 ±0.05
B. pumilus	6.5 ±0.08
B. polymyxa	6.7 ±0.03
Lactobacillus acidophillus	4.5 ±0.05
Lactobacillus plantarum	4.7 ±0.06
Lactobacillus brevis	4.3 ±0.05

Micrococcus luteus	10.5 ±0.03
Azotobacter beijerinckii	11.5 ±0.11
Pseudomonas putida	8.5 ±0.12
Pseudomonas fluorescens	10.6 ±0.07
Pseudomonas aeruginosa	10.5 ±0.08
Streptococcus salivaris	6.5 ±0.03
Staphylococcus saprophyticus	7.8 ±0.07
Erwinia amylovora	8.5 ±0.09
Enterobacter hormechei	7.6 ±0.06
Agrobacterium fabrum	11.7 ±0.12
Flavobacterium anhuiense	11.6 ±0.09
Acinetobacter soli	8.5 ±0.07
Burkholderia sp.	11.8 ±0.13
Rhizobium sp.	11.8 ±0.06
Serratia sp.	3.2 ±0.04
Nitrosomonas sp.	2.5 ±0.05
Nitrosococcus sp.	1.5 ±0.02
Nitrobacter sp.	1.3 ±0.04
Nitrospira sp.	1.7 ±0.03
Nitrosovibrio sp.	1.5 ±0.05

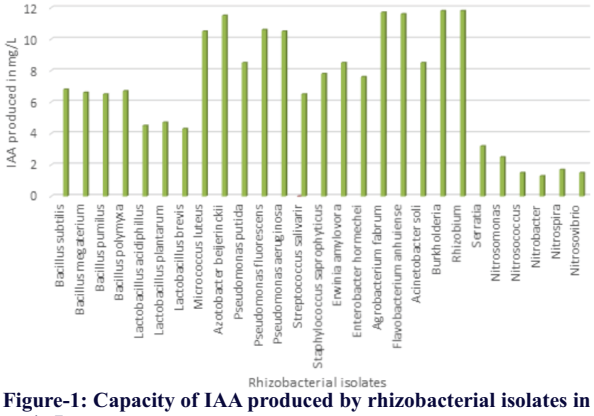

Figure-1: Capacity of IAA produced by rhizobacterial isolates in mg/mL

RESULTS AND DISCUSSION

All the rhizobacterial isolates of paddy growing fields viz., *Bacillus subtilis, B. megaterium, B. pumilus, B. polymyxa, Lactobacillus acidophillus, L. plantarum, L. brevis, Micrococcus luteus, Azotobacter beijerinckii, Pseudomonas putida, P. fluorescens, P. aeruginosa, Streptococcus salivaris, Staphylococcus saprophyticus, Erwinia amylovora, Enterobacter hormechei, Agrobacterium fabrum, Flavobacterium. Anhuiense, Acinetobacter soli, Burkholderia sp., Rhizobium sp. and Serratia sp., Nitrosomonas sp., Nitrosococcus sp., Nitrobacter sp., Nitrospira sp.* and *Nitrosovibrio sp.* showed positive IAA production capabilities at different concentrations in the presence of L-Tryptophan (Table-1; Figure-1). *Micrococcus luteus, Azotobacter beijerinckii, Pseudomonas fluorescens, Pseudomonas aeruginosa, Agrobacterium fabrum, Flavobacterium anhuiense, Burkholderia* sp. and *Rhizobium* sp. produced highest amount of IAA, in the concentration of 10.5 ±0.03, 11.5 ±0.11, 10.6 ±0.07, 10.5 ±0.08, 11.7 ±0.12, 11.6 ±0.09, 11.8 ±0.13 and 11.8 ±0.06 mg/L of IAA respectively. The amount of IAA produced by *Bacillus subtilis, B. megaterium, B. pumilus, B. polymyxa, Pseudomonas putida, Streptococcus salivaris, Staphylococcus saprophyticus, Erwinia amylovora, Enterobacter hormechei* and *Acinetobacter soli* was 6.8 ±0.06, 6.6 ±0.05, 6.5 ±0.08, 6.7 ±0.03, 8.5 ±0.12, 6.5 ±0.03, 7.8 ±0.07, 8.5 ±0.09, 7.6 ±0.06 and 8.5 ±0.07 mg/L respectively. *Lactobacillus acidophilous, L. plantarum, L. brevis* and *Serratia* sp. produced 4.5 ±0.05, 4.7 ±0.06, 4.3 ±0.05 and 3.2 ±0.04 mg/L of IAA respectively. However, the IAA produced by Nitrifying bacteria viz., *Nitrosomonas, Nitrosococcus, Nitrobacter, Nitrospira* and *Nitrosovibrio* was extremely low, about 2.5 ±0.05, 1.5 ±0.02, 1.3 ±0.04, 1.7 ±0.03 and 1.5 ±0.05 mg/L respectively (Table-1; Figure-1).

Soil microorganisms exhibit three types of association viz., neutral, beneficial or positive and detrimental or negative. The soil bacteria have the ability to increase soil yield by releasing beneficial exudates and antimicrobial compounds[12]. In the present investigation, all the rhizobacterial isolates showed positive IAA producing capacities at different concentrations. Bacteria producing high concentrations of IAA caused increase in the number of lateral roots[13]. Kloepper *et* and Mashigushi[15] reported that high concentration of IAA has low inhibition on the roots of monocotyledons. Patten and Glick[16] reported that low concentration of IAA stimulates root elongation while high concentration of IAA caused formation of lateral and adventitious roots.

The Indole pyruvic decarboxylases encoded by genes (IPdc) are the principal enzyme which determines the biosynthesis of IAA in the bacterial isolates[15]. Patten and Glick[16] have suggested that the interaction between the plant roots and bacterial IAA may be due to direct stimulation of plant cell elongation or cell division and indirectly by influencing bacterial amino-cyclopropane-decarboxylate (ACC) deaminase activity. ACC deaminase hydrolyses plant ACC which is an intermediate precursor of the phytohormone ethylene, thereby preventing the inhibition of plant growth by the level of ethylene produced[17]. The present results suggest that the plant growth promoting rhizobacteria (PGPR) have the ability to produce indole acetic acid (IAA) and promote plant growth[18-20]. The present investigations also gain support from the work of Bloembery *et al*[21] and Jetiyanon and Kloepper[22] who have investigated the role of PGPR as biofertilizer.

CONCLUSIONS:

From the results it can be concluded that the present PGPR rhizobacterial isolates sufficiently produce IAA to enhance plant growth, and may be used as an inoculants biofertilizers in rice or other cereal growing fields to replace chemical fertilizers.

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Conflict of Interest: Authors declare no any conflict of interest directly or indirectly.

REFERENCES

1. Kloepper JW, Schroth MN (1978): Plant growth-promoting rhizobacteria on radishes. In: Gilbert C (ed), *Proceedings 4th international conference on plant pathogenic bacteria*, Tours, France, pp 879–882.
2. Antoun H, Prevost D (2005): Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (ed) PGPR: *biocontrol and biofertilization*. Springer, Netherlands, pp 1–38.
3. Gray EJ, Smith DL (2005): Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. *Soil Biol Biochem,* **37:** 395–412.
4. Biswas, J.C., Ladha, L.K and Dazzo, F.B. (2000): Rhizobia inoculation improves nutrient nutrient uptake and growth of low land rice. *J. Soil Sci*., **64:**1644-1
5. Husen, E (2003): Screening of soil bacteria for plant growth promoting activities in vitro. *J.Agric. Sci*., **4:** 27-31.
6. Jeons, L.S., Kim, H., Ahn, T and Song, H. (2003): Plant growth promotion in soil by some inoculated microorganism. *Journal of Microbiology*, **41(4):**27- 276.
7. Thakuria, D., Talukdar, N.C., Goswami, C., Hazarika, S., Boro, R.C and Khan, M.R. (2004): Characterization and screening of bacteria from rhizosphere of rile grown in acidic soils of Assam. *Curr. Sci*., **86:** 978-985.
8. Belimov, A.A., Kunakova, A.M., Safronova, V.I., Stepanok, V.V., Yudkin, L.Y., Alekseev, Y.V and Kozhemyakov,A.P.(2004): Employment of rhizobacteria for inoculation of barley plantscultivated in contaminated lead and cadmium. *Microbiology*, **73:** 99-106.
9. Nemhauser, J.L., Hong, F and Chory, J (2006): Different plant hormones regulate similar processes through largely non overlapping transcriptional responses. *Cell*, **126:** 467-475.
10. Dey, R., Pal, K.K., Bhatt, D.M and Chauhan, S.M. (2004): Growth promoting and yield enhancement of peanut (Arachis hypogaea) by application of growth-promoting rhizobacteria. *Microbial. Res*., **159:** 371-394.
11. DE-Bashan, L.E and Bachan, Y. (2008): Joint immobilization of plant growth promoting bacteria and green microalgae in alginate beads as experimental model for studying plant- bacterium interaction. *Applied Environmental Microbiology*, **74:** 6798-68.
12. Alexander, M. (2000). Soil Microbiology. Eleventh edition. Wiley and Harcourt, Texas, USA. pp 58- 60.
13. Mike-Anosike, E.E., Braide, W and Adeleye, S.A. (2018). Studies on Indole Acetic Acid (IAA) Production by Rhizobacteria and Growth promoting potentials. *Int. J. Adv. Res. Biol. Sci*. **5(2):** 133-140. DOI: http://dx.doi.org/10.
14. Kloepper, J.W., Zablotowies, R.M., Tipping, E.M and Lifshits, R. (1991): Plant growth promoting mediated by a bacterial rhizosphere colonizer. In: keister, D.L., Cregain, P.B (editions), the rhizosphere and plant growth. Kluwere Academic Publishing, Dordrecht, pp 315-326.
15. Mashiguchi, K. (2011): The main auxin biosynthesis pathway in Arabidopsis. *Proc. Natl. Acad. Sci.* USA,**108 (45):**18512-18517.
16. Patten, C.L and Glick, B.R. (2002): Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*. **42:** 207-220.
17. Achard, P., Cheng, H., De Grauwe, L., Decat,J., Schoutteten, H., Moritc, T., Van derstraeten, D., Peng, J and Herberd, N.P. (2006): Integration of plant responses to environmentally activated phytohormones. *Singnals Science*, **311:** 91-94.
18. Bai, Y., Zhou, X and Mith, D.L. (2003): Enhanced soybean plant growth resulting from coinoculation of Bacillus strain with Bradyrhizobium japonicum. *Crop Sci*.,**43:** 1774-1781.
19. Ahmad, F., Ahmad, I and Khan, M.S. (2006): Screening of free living rhizospheric bacteria for their multiple growth promoting activities. *Microbial. Res*., **36:**1-9.
20. Zhao, Y. (2010): Auxin biosynthesis and its role in plant development. *Annual Review Plant Biology.***61:** 49-64.
21. Bloembery, G.V and Lugtenberg, B.J.J. (2001): Molecular basisof plant growth promotion and biocontrol by rhizobacteria. *Curr. Opin. Plant Biol*., **4:** 343-350.
22. Jetiyanon, K and Kloepper, J.W. (2002): Mixtures of plant growth promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biol. contr.*, **24:** 285-298.