Research on Rhizobacteria Production of Indole Acetic Acid (IAA) and Its Potential to Promote Growth

Pratibha Singh Chandel*

*Assistant professor, Department of Microbiology, Govt jajwalayadev naveen girl's college janjgir (Chhattisgarh)

Abstract
This study focused into how soil bacteria produce indole acetic acid. Using accepted techniques, the soil rhizospheric bacteria were isolated and characterised. Two bacterial species with varying potentials were found when rhizosphere bacteria were screened for the ability to produce IAA. The microorganisms are identified as Bacillus sp I (RSS2), Micrococcus sp I (RSS2). The benefits of inoculating maize seeds with different bacterial isolates to promote germination and growth were investigated through hydroponic research. In addition to improving germination, materialization of maize seeds encouraged the elongation of roots and shoots. The findings demonstrated that a high IAA-producing bacterial inoculation increased the number of lateral roots.

Keywords: Indole Acetic Acid (IAA), Rhizospheric soil, maize seeds, Rhizobacteria.

Introduction

According to Kloepper et al. (1993), the rhizosphere is the small area of soil that is directly impacted by root secretions and related soil microbes. The rhizosphere is home to a variety of bacteria that consume plant cell slough, protein, and carbohydrates secreted by roots. In Bashan et al. (2004). After being inoculated onto the seeds, soil bacteria known as plant growth promoting rhizobacteria (PGPR) colonise the roots of plants to promote plant development. PGPR enhanced plant growth by direct and indirect means with its specific mechanisms is not fully understood. Indole-3-acetic acid (IAA) is a heterocyclic compound containing carboxymethyl group (acetic acid) that belongs to the most studied phytohormone, and is involved innumerous mechanisms in plant physiology.

In general, auxin controls the direction, strength, and form of all organ growth as well as the interactions between them (Patten and Glick, 2002). According to Nemhauser et al. (2006), it delays fruit senescence, promotes growth and development, produces shoot epical dominance, and has a minimal impact on the development of reproductive organs and the start of flowering. The best effect of PGPR on plant growth was reduction in environmental stresses by the bacteria, providing the plant with a more favourable. Inoculation sometimes permits plant growth in soil that normally did not allow plant growth as a result of environmental stressors, which include drought, salinity, heavy metal toxicity, toxicity by other substances and suboptimal levels of nitrogen (Bacilio *et al.,* 2003). This study reports on the production of indole acetic acid by soil rhizobacteria and their growth promoting capabilities.

Materials and Methods

At Bilaspur, Panicum maximum rhizospheric dirt was gathered. Within an hour after being collected, composite soil samples were randomly collected and placed in a sterile polypropylene bag for analysis. PGPR Bacteria Isolation from Soil Sample. A sterile physiological saline serial dilution was used to analyze the soil sample microbiologically (Cheesbrough, 2002). Ten grams of soil sample was dissolved in ninety milliliters of physiological saline in a 200 ml Uniscope conical flask to obtain 101dilutions. Further dilutions were done decimally until 105were obtained. One-tenth milliliter (0.1 ml) of the various dilutions was dispensed onto freshly prepared surface dried nutrient agar medium and spread evenly with a sterile hockey-stick like glass spreader before incubating at ambient temperature (28±020C) for 48h.

Characterization and identification of soil PGPR

The isolates' pure cultures were described using colonial, microscopic, and biochemical techniques (Cheesbrough, 2002). Using the standard identification handbook (Buchanan and Gibbon, 2000), the isolates' identities were verified.

Standardization of inoculums

A loopful (6 mm) of the isolates were picked from the slants and inoculated differently into 200 mls conical flask containing 30 mls of sterile tryptone soy broth. The cultures were incubated on a rotary shaker at 150 rpm at room temperature for 24 h. The cultures centrifuged at 3000 rpm for 15 mins, was washed in physiological saline and re-suspended in sterile distilled water while optical density (O.D) of the suspension was adjusted to 0.6 at 600 nm.

Screening for IAA production

Screening for IAA production was done using Jeon’s medium as described by Jeons et al. (2003). Pure cultures of the bacterial isolates were inoculated into 5 mls of Jeon’s medium in 20ml test tubes. The tubes wee incubated at room

temperature for 72h, there after centrifuged at 3000rpm for 10 mins and the supernatant used for the screening. Two milliliters (2mls) of the each supernatant was mixed with 2 mls of Salkowski's reagent (2% of 0.5 FeCl2 in 35% perchloric acid) and incubated at room temperature in the dark for 30 mins. The presence of IAA was determined by the development of pink colour and the IAA concentration was measured spectrophotometrically at 530 nm and quantified in IAA standard curve.

Root and Shoot Elongation Assay

Root and shoot elongation assay was carried out by sterilization of the maize seed in 1% sodium hypochloride (hypo bleach). The seeds were washed repeatedly with distilled water to remove any trace of the bleach on the maize seeds. The sterilized maize seeds were inoculated with 0.1ml of 24 h old suspension of the isolates, followed by seed germination in a petri dish containing moist cotton wool. A control was set up without inoculants. The seeds bearing radicles were suspended with the aid of a needle in water contained in a beaker. Elongation of shoots and roots and number of lateral root was observed and measurement taken weekly for a period of three weeks.

Results

Identification of isolates Tables 1 and 2shows the colonial and microscopic characteristics of rhizospheric bacteria isolated from soil of Panicum maximum. The biochemical characteristics and cell morphology of the bacteria isolated is shown in Table 3.The bacterial isolates identified include species of Micrococcus, Bacillus.

Table 1 Colonial characteristics of Bacteria isolated from the soil of *Panicum maximum*on Nutrient Agar medium

Colony code	Colonial characteristics	Probable identity
RSS1	Small circular low convex and entire yellow colonies	*Micrococcus* sp
RSS2	Large serrated dull and dry flat cream colonies	*Bacillus* sp

Table 2 Microscopic and cell morphology of Bacterial isolates

Colony code	Cell morphology	Motility	Grams stain	Spore	Flagellum	Capsule	Probable Identity
RSS1	Cocci predominantly in tetrads, few in pairs	-	G+	-	-	-	*Micrococcus*sp
RSS2	Large short rods with central spores in chains	+	G+	+	+	-	*Bacillus*sp

Table 3 Biochemical and sugar fermentation

Colony code	Cat	Oxi	IN	MR	VP	Cit	Glu	Suc	Lac	Mann	Mal	Xyl	Ara	Coag	Identity of Bacterial Isolates
RSS1	+	-	-	+	-	+	-	-	-	-	-	-	-	-	*Micrococcus luteus*
RSS2	+	-	-	-	+	+	+	-	-	-	-	+	+	-	*Bacillus subtilis*

Indole Acetic Acid (IAA) Production by Bacterial isolates

Table 4 shows the concentration of IAA produced by the bacteria. All the bacterial isolates exhibite positive IAA production capabilities at different concentrations in the presence of L-Tryptophan.

Table 4 Indole Acetic Acid (IAA) Production by Bacterial isolates

Bacterial isolates	Colony code	IAA (mg/L)
Micrococcus luteus	RSS1	10.0 ±0.01
Bacillus subtilis	RSS2	5.8 ±0.05

The growth performance of PGPR bacteria in respect to shoot and root elongation and number of lateral roots. Growth performance was positive for all the PGPR bacteria compared to the control without the presence of any bacteria. Lateral root was delayed in the control until after 96hrs (fourth day). Higher shoot and root elongation was observed on maize seeds inoculated with Micrococcus luteus, although this was not significant compared to seeds inoculated with the other isolates. The maximum number of lateral roots was observed with Micrococcus luteus.

Discussion

Microorganism living in the soil exhibit many different types of association or interaction. Some of the associations are neutral; some are beneficial or positive; others are detrimental or negative. The bacterial species isolated from the rhizospheric soil of Pannicum maximum belong to the genera Bacillus and Micrococcus. They are soil borne organisms with potentials to increase soil yield by releasing beneficial exudates and sometimes antimicrobials (Alexander, 2000). The results suggested that plant growth promoting rhizobacteria (PGPR) have the potentials to produce indole acetic acid (IAA) thereby improving the growth This could therefore add to knowledge that, The abilities of all the isolates to germinate maize seedlings also proved their potentials to produce gibberellins (Cassanet al., 2009b). Reported that low level of IAA stimulates root elongation while high level of bacterial IAA production resulted to the formation of lateral and adventitious roots as was evident in the seedlings inoculated with Bacillus and Micrococcus.

Reference

1. Alexander, M. (2000). Soil Microbiology. Eleventh edition. Wiley and Harcourt, Texas, USA. pp 58- 60.
2. Ahemad, M. & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univ-Sci,* 26(1), 1–20.
3. Bashan, Y., Holguin, and de-Bashan, L.E. (2004). Azospirillum- plant relationships: physiological, molecular, agricultural and environmental advances. Can. *J. Microbiol.,* 50:521-577.
4. Bacilio, M., Vazquez, P and Bashan, Y, (2003). Alleviation of obnoxious effects of cattle ranch compost on wheat seed germination by inoculation with Azospirillium spp. Boil. *Fertile. Soils*, 38:26- 266.
5. Cheesbrough, M. (2002). District Laboratory Practice in Tropical Countries Part Two. Low price edition, Cambridge University Press, UK. pp 117.
6. Cassan, F., Perrig, S.V., Masciarelli, O., Penna, C and Luna, V. (2009b). Azospirilliumbrasilense Az39 and Bradyrhizobiumjaponicum E 109, inoculated singly or in combination, promote seedgermination and early seedling growth in Zea mays and Glycine max. *Eur. J. Soil Biol*. 45:28-35.
7. Kloepper, J.W. (1993). Plant Growth Promoting Rhizobacteria as Biological Control Agents in Soil Microbial Ecology: Application in Agricultural and Environmental Management F.B. metting,Jr., edition, Marcel DekkerInc. New York,USA.pp 255- 274.
8. Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J Soil Sci Plant Nutr., 13(3), 638–649.
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. Patten, C.L and Glick, B.R. (1996). Bacterial biosynthesis of indole-3acetic acid. *Canadian Journal of Microbiology*. 42:207-220.
10. 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.
11. Vaghasiat, H.L., Patel, G.M., Chudasama, R.S., Bhott, K.R. 2011. Screening of IAA from rhizospher microflora of field crops. *Bioscience Discovery*. 02, 94-100.
12. Wahyudi, A.T., Astuti, R.P., Widyawati, A., Meryandini, A., Nawangsih, A.A. 2011. Characterization of Bacillus sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting Rhizobacteria. *Journal of Microbiology and Antimicrobials.* 3, 34-40.
13. Gupta S, Kaushal R, Sood G (2018) Impact of plant growth–promoting rhizobacteria on vegetable crop production. *Int J Veg Sci* 24(3):289–300.