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Isolation and characterization of IAA producing plant growth promoting rhizobacteria (PGPR) from rhizospheric soil of ornamental (Marigold) plant.

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ABSTRACT

Indole acetic acid (IAA) production is a major property of rhizosphere bacteria that stimulate and facilitate plant growth. The present work deals with isolation, characterization and identification of indole acetic acid producing bacteria from the rhizospheric soil of marigold plant. The isolated bacterial strains are *Azotobacter, Pseudomonas* and even *Spirillum* (Gram negative bacteria); which show positive test in IAA, catalase, oxidase, urease and starch hydrolysis testing respectively. The bacteria strain has capacity to produce 3.8mM concentration of IAA from L-tryptophan. Again, IAA treated marigold seeds showed more root and shoot growth in comparison to control. So, these bacteria can be good source of IAA and can be used as alternatives to fertilizers.

Key words: PGPR, IAA, Marigold plant, Biochemical test.

INTRODUCTION

PGPR (Plant Growth-Promoting Rhizobacteria) can produce Indole acetic acid (IAA) from the metabolism of L-tryptophan. IAA is one of the most physiologically active auxins. PGPR can influence plant growth by producing plant growth regulators like auxin, gibberellin, and ethylene (Arshad *et al.*, 1992). IAA helps in the formation longer root with branched root hairs which are involved in nutrient uptake (Datta and Basu, 2000). Bacteria synthesize auxins in order to control host physiological processes for their own benefit (Shih-Yung, 2010). Bacteria have more than one pathway for the formation of IAA from tryptophan (Pattern and Glick, 1996). *Azospirillum brasilense could* produce IAA in the absence of tryptophan when grown aerobically (Horemans and Vlassak, 1985). Here tryptophan independent pathway might contribute significantly to the newly synthesized IAA; however, extensive Trp-to-IAA conversion also occurs in such preparations. The first objective of this study was to isolate and screen indigenous Indole acetic acid producing bacteria from different rhizospheric soil. The second was to purify the IAA and screen their

abilities of plant growth promoting rhizobacteria attributes. Besides, optimization study intended for high IAA production was carried out with physicochemical parameters such as carbon and nitrogen source, with and without supplement of tryptophan, pH and temperature.

MATERIALS AND METHODS

Isolation of IAA producing bacteria

Soil sample was collected from rhizospheric region of marigold plant. 1g of rhizosphere soil was taken in test tube and 9 mL sterile distilled water was added with it. 1ml sample was serially diluted up to 10^{-6}. 0.1 mL of diluted sample was plated on sterile potato dextrose agar (PDA-media) and incubated for 3 days at 28 °C. Single colonies were picked up and streaked on sterile PDA agar plates to get pure culture. Well isolated colonies were observed for morphological characterization. The isolates were further checked for IAA production.

Identification and biochemical characterisation of bacteria

The isolates based on micromorphological observation and biochemical characterization were identified. The tests involved, were Gram staining, Catalase test, Oxidase test, Urease test, starch hydrolysis test etc. (Aneja, 2001).

Quantification of IAA

IAA was quantified by spectrophotometric method. 1.5 mL of peptone water was centrifuged in 10,000 rpm for 10 minutes at 4^0C. 1mL of supernatant was collected and added with 1 mL of Salkowski reagent solution. It was mixed and stored for 30 minutes at room temperature in dark place. Absorbance was taken at 540nm by using visible spectrophotometer. Standard graph was drawn by taking concentration of stock solution. The concentration of IAA produced from isolates was determined by putting the value of sample solution (peptone water containing isolates and Salkowski reagent) in standard graph.

Effect of IAA producing isolates on plant growth

To study the effect of IAA producing rhizospheric isolates on plant growth, pot assay was performed. Marigold seeds were used for these purposes. The pot content was the mixture of 50% garden soil, 30% organic compost and 20% sand. Pots were marked as two categories i.e. control and treated pots. Two seeds were sown in each pot. Pots were irrigated with sterile distilled water every day and kept in shade-net house condition. Plant was uprooted and seedlings were measured for shoot and root length after 15th day.

RESULT

Isolation and Identification of rhizospheric

Bacterial isolate was identified as gram negative during Gram staining method. Again red colouration, effervescence, decolouration, purple pink colour and clear zone indicated that IAA testing, catalase test, oxidase test, urease test and starch hydrolysis test become positive respectively. Bacterial isolates were successfully isolated as IAA producer from rhizosphere soil. Bergey's manual of determinative of bacteriology was used as a reference to identify the isolates (MacFaddin, 2000). The isolate was identified as gram negative bacteria i.e. Azotobacter, Pseudomonas and even Spirillum.

Characterization of IAA production potential

IAA production was checked with use of Salkowski reagent. Colour development was first visible at the highest IAA concentration within minutes and continued to increase in intensity for a period of 30 min (Figure 2). Hence optical density was measured after 30 and 120 min. If colour development was not observed after 30 min, it was not kept for further incubation up to 120 min. The results were in support to previous study (Ghosh and Basu, 2002). From the standard graph, the concentration of IAA producing bacterial isolate was recorded as 3.8mM (Figure 1).

The change in colour of methylene blue to colourless within few seconds determined the positive oxidase test for isolate (Figure 3). The purple pink colour urease broth indicated positive reaction for urease test (Figure 4). Effervescence was found due to break down of H_2O_2 into H_2O and oxygen determined positive catalase test for isolate (Figure 5). Again, clear zone was found around the bacterial colonies determine the presence of amylase enzyme show positive starch hydrolysis test (Figure 6).

Morphological parameters of marigold plants

The length of root and height of the marigold plant in treated pot were 4cm and 10cm respectively which were greater than control (2cm and 8 cm respectively).

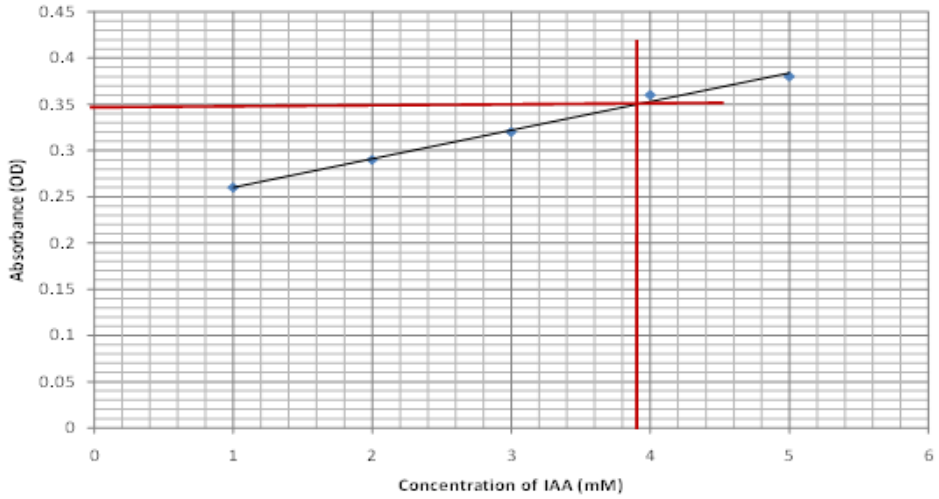

Fig. 1 : Graph for quantification of IAA

Fig. 2 : IAA testing

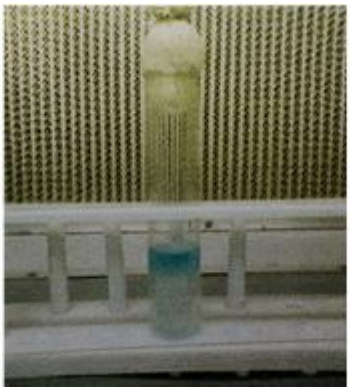

Fig. 3: Oxidase test

Fig. 4: Urease test

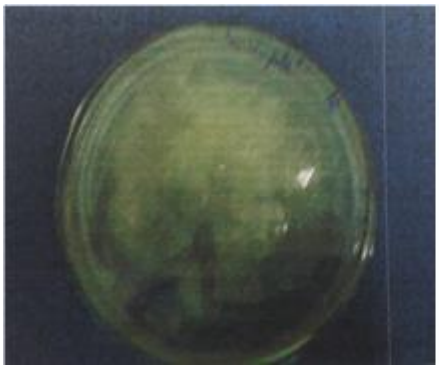

Fig. 5: Catalse

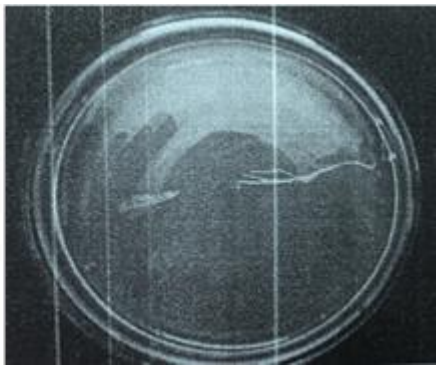

Fig. 6 Starch hydrolysis test

DICUSSION

IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. Most of studies from the earlier work showed that IAA producing organisms are Gram negative (Lindow *et al*., 1998; Datta and Basu, 2000). Few Gram positive strains belong to Bacillus strain known to produce IAA (Wahyudi *et al.*, 2011). Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1992). The amount of IAA produced by the bacteria was within the detection limits of Salkowski reagent (Ehmann, 1977). The reagent gives reaction with IAA and does not interact with L-tryptophan and used in large (Vaghasiat *et al*., 2011). The property of synthesizing IAA is considered as effective tool for screening beneficial microorganisms suggesting that

IAA producing bacteria have profound effect on plant growth (Wahyudi *et al*., 2011). Present study showed that IAA positive strains were Gram negative and show positive IAA test, oxidase test, urease test, catalase test and starch hydrolysis test respectively.

Inoculation with IAA producing bacteria induces the proliferation of lateral roots and root hairs. Fatima *et al.* (2009) also showed that germination rate, roots, shoot growth of plant were increased by IAA and PGPR. There was increase in root and shoot elongation and determine positive effect on plant growth and thus can be considered as plant growth promoter.

CONCLUSION

From this study, it is clear that rhizospheric soil can provide a rich source of IAA producing bacteria and has the ability to produce a significant amount of IAA in a tryptophan-supplemented medium. We conclude these rhizobacteria have capacity to produce IAA. This can induce root growth in Marigold plants (ornamental plants). So Spirillum, Azotobacter and pseudomonas volutans can be a good alternative to IAA and can prevent environmental pollution by avoiding excessive applications of industrially produced fertilizers to cultivated fields.

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