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# Production of Indole Acetic Acid from soil bacteria: A alternative approach on plant growth

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## Abstract

Indole acetic acid (IAA) production is a major assets of rhizosphere bacteria that stimulate and enhance plant growth. The present work deals with isolation and identification of indole acetic acid producing bacteria from the rhizospheric soil collected from Pomegranate, Guava and Amla farm. Out of ten Indole acetic acid producing isolates, two were selected as efficient producers. Spectrometric analysis of IAA was done after 24, 48 and 72 hours of culture which showed that the isolated bacteria produced maximum concentration of IAA after 72 hours of incubation period at 37°C. The concentration was measured using a standard IAA curve and the maximum concentration was obtained by AA2. Subsequently, effect on plant growth was tested by pot assay. The in vitro treatment of pea seeds with AA2 isolate for germination showed better result than control. In conclusion the study suggests the IAA producing bacteria as efficient inoculants to promote plant growth.

**Keywords:** rhizobacteria, IAA, pot assay plant growth

## Introduction

Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L- tryptophan metabolism produced by several microorganisms including Plant Growth Promoting Rhizobacteria (PGPR) (Lynch, 1985).

Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as PGPR. PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (like auxin, gibberellin, and ethylene), siderophores, HCN and antibiotics (Arshad et al., 1992). Bacteria synthesize auxins in order to perturb host physiological processes for their own benefit (Shih-Yung, 2010).

The microorganisms isolated from rhizosphere region of various crop have ability to produce Indole acetic acid as secondary metabolites due to rich supply of substrates. Indole acetic acid helps in the production of longer roots with increased

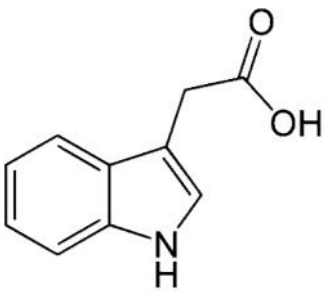
number of root hairs and root laterals which are involved in nutrient uptake (Datta et al., 2000).

IAA stimulates cell elongation by modifying certain conditions like, increase in osmotic contents of the cell, increase in permeability of water into cell, decrease in wall pressure, an increase in cell wall synthesis and inducing specific RXA and protein synthesis and it induce flowering and fruiting. (Zhao, 2010).

IAA is a metabolite derived from Trp by many Trp -dependant and Trp-independent pathways in plants and bacteria. More than one pathway could be present in a bacterium (Pattern, et al. 2002). In Trp-dependant pathway, tryptophan is converted to indole-3- acetamide (IAM) by tryptophan-2-monooxigenaseand IAM is metabolized to IAA by IAM hydrol(Matsukawa et al.,2007)

A preliminary study indicated that these growthpromoting bacteria stimulated the development of root systems. In fact, many groups have reported an association of indole-3-acetic acid (IAA) with PGPR. It is known that appropriate concentrations of exogenous IAA stimulate the growth and development of plant root systems. Some active PGPRs in peatland provide IAA or IAA agonists, which activate root branching and lateral root.

The objective of present study is to isolate Indole Acetic Acid (IAA) producing bacteria from soil and to see the effect of Indole acetic acid (IAA) on plant growth.




## Material and Methodology

**Sample collection**: The soil samples of Pomegranate, Amla and Guava was collected from farm and used as experimental sample. The experiment was conducted in Institute of Biosciences and Technology, MGM University, Chh. Sambhajinagar during 2023-24.

**Isolation of IAA producing bacteria from rhizospheric soil:** Soil samples of each Pomegranate, Guava and Amla were diluted in three different test tubes containing sterile distilled water. The Luria broth was prepared as a growth medium for cultivation of rhizosperic bacteria present in soil sample. The broth was autoclaved in three different flasks, then the diluted soil sample were inoculated in the broths. This culture was incubated for 48hrs at 30°C.After 48 hrs enriched soil sample were spread on Abhay's agar .Theses agar plates were incubated for growth of bacterial colony.

**Determination of IAA:** To determine the amounts of IAA produced by each isolate, a colorimetric technique was performed with the Salkowski's method. The isolates were grown in yeast malt dextrose broth (YMD broth) (Himedia, India) and incubated at 28 °C for 4 days. The broth was centrifuged after incubation. 1ml of supernatant was mixed with 2ml of Salkowski's reagent (2% 0.5 FeCl3 in 35% HCLO4 solution) and kept in the dark. The optical density (OD) was recorded at 530 nm after every 30min. Isolates showing pink to red colour were selected as IAA producers and were used in further experiments. The amount of IAA was measured by spectrophotometric method at 535 nm. Then concentration was calculated using standard curve of IAA. The concentration of bacteria was measured by using standard curve at a range of 0.5-10 µg IAA (Sigma-Aldrich).

**Identification and characterization of bacteria**: The bacterial isolates isolated on Abhays agar were identified based on micro morphological observation and biochemical characterization.

**Production and Extraction Of IAA**: Those isolates producing high amount of indole acetic acid was inoculated in YMD broth and it was centrifuged at 15000 × g for 15 min. The supernatant was collected and mixed with ethyl acetate (1: 2). After vigorous shaking it was allowed to stand for 10 min. IAA was extracted within solvent layer. The procedure was repeated 3 to 4 times.

**Effect of IAA on growing plant:** To study the effect of IAA producing rhizospheric isolates on plant growth, pot assay was performed. Local Pea seeds were used for seed coating. The Pea seeds were surface sterilized by immersing in 95% ethanol for 30 s. The disinfected seeds were washed 5 times by sterile distilled water. 0.1ml overnight grown culture (0.5 OD) was applied on seed surface for seed coating. Seeds were dried and sowed into sterile soil as carrier. Total 5 seeds were sown in pot used per pot at equal distance and experiment was performed in triplicates for each isolates. The uncoated seeds were used as control .After appearing seedlings of soil 0.1 g of Trp per kg soil after being solving in water was added to every pot. Pots were irrigated with sterile distilled water every day and kept in sunlight. At the interval of every 5th day, plant was uprooted and seedlings were measured for shoot and root length and chlorophyll content up to 15th day.

## Results and Discussion

### Isolation of IAA producing bacteria from rhizosphereic soil

All three soil rhizosphere samples showed positive bacterial growth on medium. From the plates 10 independent bacterial isolates were collected according to their cultural and morphological characteristics i.e. by visual observation of the isolates followed by periodic subculture on nutrient agar plate. These plates were designated as AA1 to AA-10. Two out of 10 isolates exhibited positive reaction by forming prominent pink solution when reacting with Salkowsi reagent. Thus they are considered as potentially IAA producing isolates. These isolates were AA 4 and AA-2.

### Quantitative determination of IAA production from bacteria

The use of the technique for the detection of IAA using the Van Urk Salkowski reagent is an important option for qualitative and semi-qualitative determination that assure the presence of the hormone in the supernatant of bacterial cultures or liquid formulations of biological inoculants. 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 Na-acetyl-L-tryptophan and used by and large (Vaghasiat *et al.*, 2011). Among the isolates AA-2 and AA-4 were found to be the best producer of IAA.

AA-2 and AA-4 were able to produce moderate to high amount of IAA under laboratory condition. During the course of 72 hrs incubation, IAA production increased with time. Highest IAA concentration was observed with AA-2 (10µg/ml) followed by AA- 4 (7ug/ml) in a 72hrs incubation scheme.

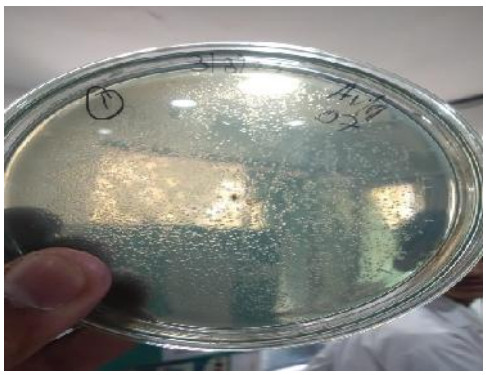


Fig 1 Rhizopheric bacteria on Asbays medium

Fig.2 Production of IAA

Fig. 3 Extraction of IAA

## Characterization of bacteria:

AA2 and AA-4 isolates were subjected to morphological and biochemical characterization. Cellular Shape and Gram staining were performed for morphological characterization and the findings are presented in Table 1. The biochemical characteristics of bacterial isolates are summarized in Table 2.

All ten isolates are positive for IAA production but among those two isolates AA-2 and AA-4 were selected as potential IAA producers. The earlier work showed that IAA producing organisms are Gram negative (Lindow *et* al., 1998; Datta and Basu, 2000).

## Morphological characterization

**Table 1: Morphological character of AA2& AA4**

| Isolates | Gram Staining | Shape |
|---|---|---|
| AA2 | Gram Negative | Rods |
| AA4 | Gram Negative | Rods in chain |

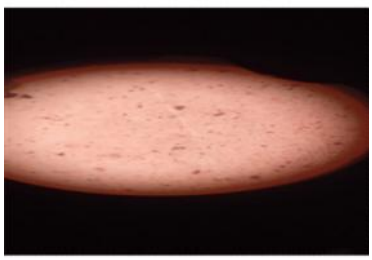


Fig.4 Grams nature of AA2

## Biochemical characterization:

**Table 2: Biochemical characterization of AA2& AA4**

| Biochemical properties | AA2 | AA4 |
|---|---|---|
| Oxidase | Negative | Positive |
| Catalase | Negative | Positive |
| Indole | Positive | Negative |
| Methyl red | Negative | Negative |
| VogesProskauer | Positive | Negative |
| Citrate | Positive | Positive |
| Sugar Fermentation Test Glucose | Positive | Positive |
| Lactose | Positive | Positive |
| Sucrose | Positive | Positive |
| $H_2S$ production | Negative | Negative |
| Urease | Negative | Negative |
| Starch hydrolysis | Positive | Negative |

## Effect of Indole acetic acid on plant growth:

The indole acetic acid (IAA) extracted in laboratory was more helpful and it was found that growth was enhanced towards the plant. The pea beans coated with IAA produced healthy and anti-diseased plants. Whereas the control plant`s growth was very slow as compare to extracted IAA from AA2 isolate.

The rhizosphere soil isolates AA-2 were significantly enhance the plant height and root length of pea beans seedlings along with increase in chlorophyll content when compared with control . In previous report, root elongation was found to occur in *Sesbania aculeata* by inoculation with *Azotobacter* spp. and *Pseudomonas* spp., in *Brassica campestris* by *Bacillus* spp (Ghosh *et al.*, 2003), and in *Pennisetum americanum* by *Azospirillum brasilense* (Tien *et al.*, 1979). This confirms the involvement of bacterial isolates AA-2 in enhancing the plant growth by synthesizing IAA.


Fig 5. Effect of IAA on plant growth

## Conclusion

IAA is the main auxin in plants, regulating growth and developmental processes such as cell division and elongation, tissue differentiation, apical dominance, and responses to light, gravity, and pathogens. Roots are most sensitive to fluctuations in IAA level. IAA stimulates overproduction of root hairs and lateral roots in plants and release of saccharides from plant cell walls during the elongation. Saccharides are a source of nutrients for microorganisms and can increase the colonization ability of plant-associated bacteria. On a larger scale, IAA serves as signalling molecule necessary for development of plant organs and coordination of growth.

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