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## Indole acetic acid (IAA) production by different bacterial sample isolated from soil of different districts of Uttar Pradesh

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**Abstract**

Indole acetic acid (IAA) production is a major property of soil bacteria namely *Pseudomonas* and *Bacillus*. The present work deals with indole acetic acid producing bacteria from the soil of different district of Uttar Pradesh. Optimization of indole acetic acid production was carried out at different cultural conditions of pH and temperature with varying media components such as carbon and nitrogen source, tryptophan concentration. In conclusion the study suggests the IAA producing bacteria as efficient biofertilizer inoculants to promote plant growth.

**Keywords:** Indole acetic acid, auxins, gram positive and soil texture

**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) [6]. 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 and Frankenberger, 1992) [1]. Bacteria synthesize auxins in order to perturb host physiological processes for their own benefit (Shih-Yung, 2010) [8]. The microorganisms isolated from rhizosphere region of various crop have an 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 and Basu, 2000) [3]. 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. It promotes embial activity, inhibit It promotes embial activity, inhibit or delay abscission of leaves, induce flowering and fruiting. (Zhao, 2010) [10]

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. Physiological evidence for different Trp-dependent pathways for synthesis in *Azospirillum brasilense* has been reported (Carreno-Lopez *et al.*, 2000) [2]. In Trp dependant pathway, tryptophan is converted to indole-3-acetamide (IAM) by tryptophan-2-monooxygenase and IAM is metabolized to IAA by IAM-hydrolase. Horemans and Vlassak (1985) [4] demonstrated that *A. brasilense* could produce IAA in the absence of tryptophan when grown aerobically showed that the highest levels of auxin were produced in the presence of NH<sub>4</sub>. It appears to be of particular importance during embryogenesis, when fine control over low levels of IAA is critical to polar development. Trp-independent pathway might contribute significantly to the newly synthesized IAA; however, extensive Trp to IAA conversion also occurs in such preparations. The objective of this study was to screen indigenous Indole acetic acid producing bacteria from different rhizospheric soil of different district of Uttar Pradesh.

**Materials and Methods****Isolation of IAA producing bacterial isolates**

Soil samples were collected from twelve district of Uttar Pradesh namely, Azamgarh, Baharaich, Balia, Chandauli, Faizabad, Jaunpur, Kannauz, Kanpur, Meerut, Mirzapur, Sultanpur and Unnaw.

The soil texture was slit, pH in the range of 7.5 to 8.5 and temperature was 30 to 32 °C. The isolation of the microorganisms was done as follows. 10g of rhizosphere soil in 250mL flask was taken and 90 ml sterile distilled water was added. It was incubated on rotary shaker at 120 rpm for 10 min. 1ml sample was serially diluted upto  $10^{-7}$ . 0.1 mL of diluted sample was plated on sterile Luria Bertani (LB) agar medium (Himedia, India) and incubated for 3 days at 28 °C. Single colonies were picked up and streaked on sterile LB agar plates to get pure culture. Well isolated colonies were observed for morphological characterization. Total 12 bacterial isolates were obtained from twelve district of Uttar Pradesh. The isolates were further checked for IAA production.

### Characterization of IAA production

To determine the amounts of IAA produced by each isolate, a colorimetric technique was performed with Van Urk Salkowski reagent using the Salkowski's method (Ehmann, 1977). The isolates were grown in yeast malt dextrose broth (YMD broth) (Himedia, India) and incubated at 28 °C and screening at 12 hour, 24 hour, 48 hour, 72 hour and 1 week. IAA production was compared in YMD and LB media.

### Result and Discussion

IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. All twelve isolates are positive for IAA production. Most or studies from the earlier work showed that IAA producing organisms are Gram negative (Lindow *et al.*, 1998; Datta and Basu, 2000) <sup>15, 31</sup>. Few Gram positive strains belong to Bacillus strain known to produce IAA (Wahyudi *et al.*, 2011) <sup>19</sup>. Present study showed that five IAA positive strains were Gram positive It has been reported that IAA production by bacteria can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability. Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1992) <sup>17</sup>.

Based on the isolation of 12 isolates of *Pseudomonas* from the soil sample of different district of U.P. *Pseudomonas* (Bacteria) were easily and rapidly grow on Luria Bertani (LB) agar medium and visualized pale yellow colour colony and Based on the isolation of 6 isolates of *Bacillus* from the soil sample of different district of U.P. *Bacillus* (Bacteria) were

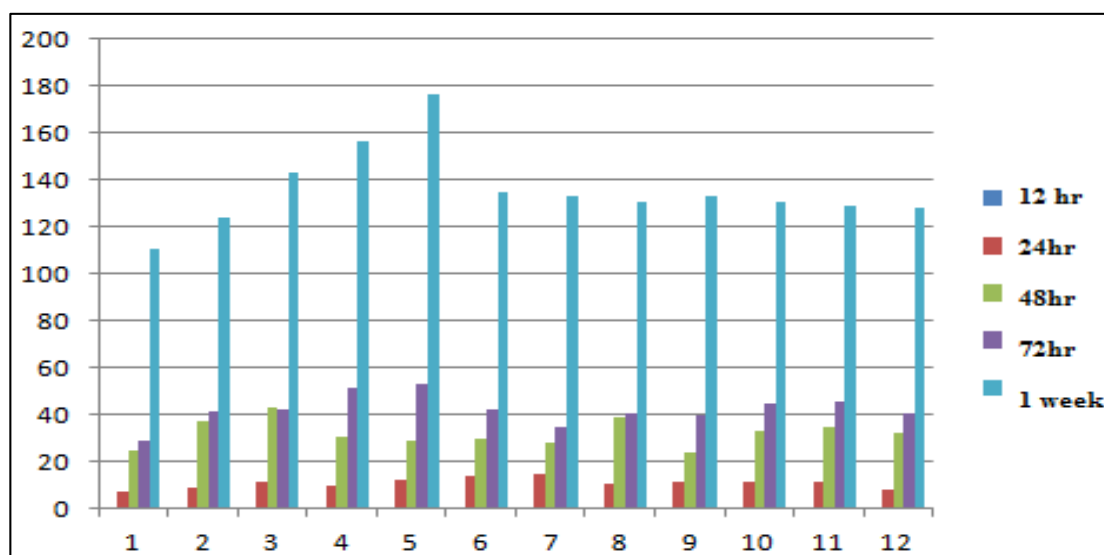
easily and rapidly grow on Luria Bertani (LB) agar medium and visualised milky white colour colony on PDA media. *Bacillus* is a genus of gram-positive, rod-shaped bacteria and a member of the phylum Firmicutes. 12 bacterial isolates of *Pseudomonas* and 12 bacterial isolates of *Bacillus* from each district were successfully isolated as IAA producer from soil. After 1 week highest IAA production of *Pseudomonas* and *Bacillus* showed in Faizabad. In 12 bacterial isolates of *Pseudomonas* showing highest IAA production observed after 12 and 24 hour in Kannauz, at 48 hour in Balia and 72 hour and 1 week in Faizabad (Table 1 and Fig 1). In 12 bacterial isolates of *Bacillus* showing highest IAA production observed after 12 hour in meerut, after 24 hour in Kannauz, at 48 hour in Balia and 72 hour and 1 week in Faizabad (Table 2 and Fig 2).

**Table 1:** IAA production of Bacterial isolates (*Pseudomonas*)

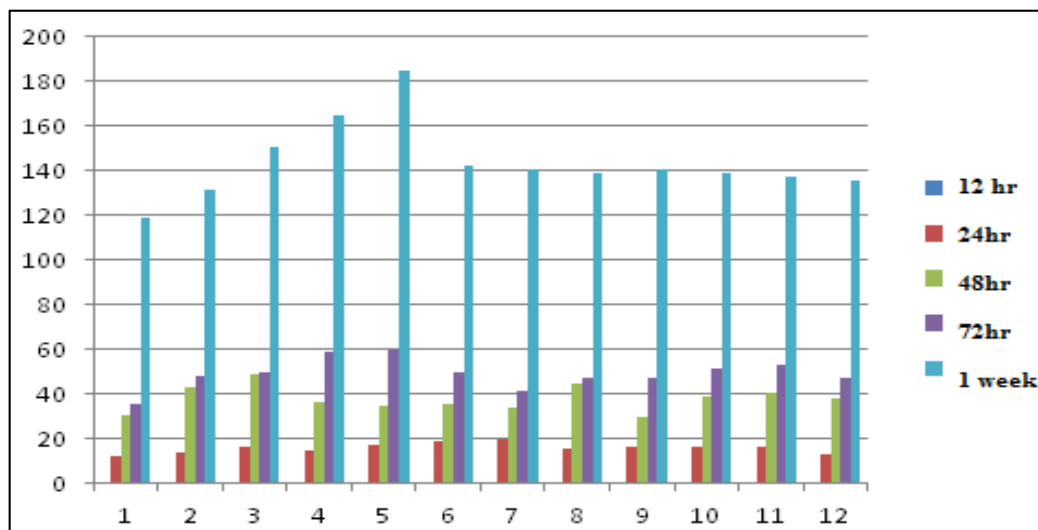
District	12h	24h	48h	72h	1 week
Azamgarh	0.002	7.567	24.879	28.564	110.564
Baharaich	0.001	8.654	36.897	41.212	123.654
Balia	0.003	10.987	42.875	42.542	142.754
Chandauli	0.002	9.996	30.543	51.543	156.876
Faizabad	0.003	11.765	28.876	52.654	176.875
Jaunpur	0.002	13.876	29.532	42.345	134.567
Kannauz	0.004	14.876	28.003	34.563	132.786
kanpur	0.002	10.876	38.765	40.453	130.876
meerut	0.002	11.543	23.658	39.876	132.786
mirzapur	0.003	11.234	32.876	44.567	130.987
sultanpur	0.001	10.987	34.654	45.765	129.098
unnaw	0.001	7.645	32.432	40.324	127.876

**Table 2:** IAA production of Bacterial isolates (*Bacillus*)

District	12h	24h	48h	72h	1 week
Azamgarh	0.006	12.567	30.879	35.564	118.564
Baharaich	0.004	13.654	42.897	48.212	131.654
Balia	0.007	15.987	48.875	49.542	150.754
Chandauli	0.008	14.996	36.543	58.543	164.876
Faizabad	0.007	16.765	34.876	59.654	184.875
Jaunpur	0.005	18.876	35.532	49.345	142.567
Kannauz	0.007	19.876	33.654	41.563	140.786
Kanpur	0.008	15.876	44.765	47.453	138.876
Meerut	0.009	16.543	29.658	46.876	140.786
Mirzapur	0.008	16.234	38.876	51.567	138.987
Sultanpur	0.009	15.987	40.654	52.765	137.098
Unnaw	0.008	12.645	38.432	47.324	135.876



**Fig 1:** IAA production of Bacterial isolates (*Pseudomonas*)



**Fig 2:** IAA production of Bacterial isolates (*Bacillus*)

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