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ESTIMATION OF PLANT GROWTH HORMONE (IAA) PRODUCTION ABILITY BY *BRADYRHIZOBIUM* STRAINS ISOLATED FROM ROOT NODULES OF PIGEON PEA (*CAJANUS CAJAN*)

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ABSTRACT

Indole-3-acetic acid (IAA) is the common natural auxin which extensively affects pants physiology. Tryptophan (L-trp) is physiologically a precursor of auxin biosynthesis in higher plants and microorganism. Fifteen bacterial isolates in this study were screened for IAA production out of which thirteen isolates showed the ability to produce indole acetic acied. All the thirteen bacteria were of the *Bradyrhizobium spp.* Significant variation was observed in IAA production by different isolates ranging from 8.22-52.81 µg/ml after 48 hours and 9.35-70.045 µg/ml after 72 hours in presence of tryptophan.Potent IAA producers after 72 hours were identified as S6 (70.05 µg/ml), S4 (67.05 µg/ml), S2 (65.17 µg/ml), S3 (62.18 µg/ml), S9 (61.05 µg/ml) followed by S13 (57.68 µg/ml), S7 (45.69 µg/ml), S14 (44.19 µg/ml), S11 (38.57 µg/ml), S10 (36.70 µg/ml), S8 (33.33 µg/ml) & S1 (9.35 µg/ml) in the decreasing order. the *Bradyrhizobium* strains identified as the IAA producer may be developed as bioinoculant or biofertilizer to aid in the growth and development of the crop plants. These strains will not only help in plant growth & development but also will contribute in enhancing root/shoot growth and seedling vigor, cell elongation and cell division subsequently aiding in plant growth and development.

Introduction

Rhizobial bacteria are the best plant growth promoting among rhizobacteria. The bacteria increase plant growth and yield by various methods. Some PGPR strains enhance the growth and development of plants by interfering the concentration of known phytohormones (Kravchenko *et al*., 1994; Leinhos 1994). Among plant hormones, auxins play an important role in root system development and lastly, plants yield. Auxins play an important role in elongation of plant cells, tropism apical dominance, root formation and root elongation and lastly, promotion of ethylene production subsequently evolution and ripening fruits. Some studies shows that auxins play key role in creating nodule in leguminous plants and generally, in establishing a symbiotic association with rhizobia. Therefore, those bacteria which affect growth and development by producing indole acetic acid phytohormone (IAA) led to development of plant root system and subsequently enhancement in nutritional uptake by plant.

Indole -3-acetic acid (IAA) is the common natural auxin which extensively affects pants physiology(Neeru *et al.,* 2000). Tryptophan (L-trp) is physiologically a precursor of auxin biosynthesis in higher plants and microorganism (Spaenpen *et al.,* 2007; Tien *et al.,* 1979). In order to produce auxin, the bacteria use the amino acid tryptophan as precursor (Tien *et al.,* 1979; BelimOv *et al.,* 2001). This substance can be converted to IAA by soil beneficial bacterial activities. The root exudates are the main resource of tryptophan in soil. Various reports showed that tryptophan derived microbial auxins present in the rhizosphere area play a significant role in growth and development of plant rooting system and crop yield (Sarwar and Frankenberger, 1994; Vanloon and Bakker, 2003; Riov and Yang 1989). There are the various reports about production ability of phytohormones by diazotrophic PGPR bacteria including bacteria of genera Azotobacter (Wendo *et al.,* 2002), Azospirillum (Yasmin *et al.,* 2007) and also rhizobial bacteria(Arshad and Frankenberger, 1998). Many of rhizobial species are able to produce IAA.

Hence with this hypothesis that IAA-producing rhizobial bacteria have the ability to enhance the plant root contact surface with soil and subsequently the increase of nutrient uptake via root elongation and plant rooting system increase, the present study was scheduled to estimate the IAA production ability of the bacterial strains isolated from the root nodules of pigeon pea plants.

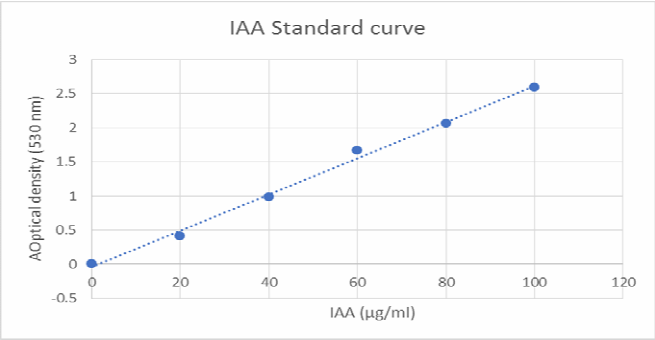
Materials and Method

Root nodule samples of 60 days old pigeon pea plants were obtained from the Begusarai, Vaishali, Samastipur districts of Bihar; Mau, Gazipur, Mirzapur & Varanasi districts of Uttar Pradesh. Root nodule samples were kept in sterile polythene bags and brought to laboratory for isolation of *Rhizobia*. The selective medium yeast extract mannitol agar (YEMA) was used for isolation of rhizobia (Vincent JM 1970) and a pure culture of each isolate was prepared after sub culturing on the same medium (YEMA). Pure cultures were authenticated as rhizobia through their nodulating ability on homologous hosts by plant infection tests (Vincent, 1970) and 16s rRNA sequencing.

For screening of the IAA production ability, bacterial isolates were grown in Luria Bertani broth supplemented with 0.01% tryptophan and incubated at 28±2˚ C for 3 days under shaking condition. The broth was then centrifuged at 10,000 rpm for 20 min at 4˚ C to collect the supernatant. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski's reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution). Development of red pinkish colour indicates IAA production (Gordon and Paleg, 1961). Absorbance was taken at 530 nm.

Standard curve of IAA

Standard curve was made by using IAA solution in 0-100 µg/ml concentration. After making volume to 1ml using distilled water followed by adding Salkowski's reagent (2 ml), total volume was made to 4 ml and incubated for 25 minutes at room temperature. Standard curve was plotted with the different readings obtained by taking absorbance at 530 nm.



Result and Discussion

Fifteen bacterial isolates in this study were screened for IAA production. Significant variation (Table 1) was observed in IAA production by different isolates ranging from 20.21 µg/ml (S5) to 72.67 µg/ml (S6) after 48 hour and 31.08 µg/ml (S11) to 82.78 µg/ml (S6) after 72 hour in presence of tryptophan. Potent IAA producers after 72 hours were identified as S6 (82.78 µg/ml), S15 (76.04 µg/ml), S9 (75.66 µg/ml), S3 (72.67 µg/ml), S12 (70.04 µg/ml), S1 (69.67 µg/ml) and S13 (64.42 µg/ml). After 48 hours, highest IAA producers were S6 (72.67 µg/ml), S15 (67.42 µg/ml), S3 (67.05 µg/ml), S9 (68.55 µg/ml), S12 (65.18 µg/ml), S1 (62.55 µg/ml) and S13 (55.43 µg/ml) (Table 2, Figure 1 & Plate 1). In all the strains, the conc. of IAA production increased from 48 hours to that in 72 hours. The rate of increase in concentration from 48 to 72 hours was highest in S5 but the concentration of IAA produced by S5 was low. Among the potential IAA producers, rate was slower in S3 & S12 which indicates that its peak production was at 48-hour duration and then after the IAA production by these strains became slower which might be due to the release of IAA degrading enzymes such as IAA oxidase and peroxidase. In all the potent IAA producing strains, the rate of increase in IAA production from 48 hours to 72 hours was less than 20%.This has been reported by Datta and Basu, (2000) that IAA production reached maximum at 72 h and then decreased. The decrease in IAA level might be due to the release of IAA degrading enzymes such as IAA oxidase and peroxidase as has been reported earlier in *Rhizobium* sp. from *Cajanus cajan* (Datta and Basu, 2000). The least IAA producing strain might be due to the presence of higher level of these enzymes leading to the nondetectable amount of IAA in the roots (Ghosh *et al.,* 2015). The production of IAA by nodule bacteria from nodular tryptophan and its implication for nodule development are well-documented [Datta and Basu, 2000; Ghosh and Basu, 2006; Ghosh *et al* 2013]. A number of *Rhizobium* isolates have been reported to produce high concentration of IAA when grown in medium containing tryptophan in culture (Tsavkelova *et al.,* 2005). Dutta and Basu (2000) also reported the IAA production by the *Bradyrhizobium* spp. isolated from the pigeon pea root nodules. Thus, the result of this study is similar to the finding of the previous studies about the IAA production ability of *Rhizobium spp*..

Thus, the *Bradyrhizobium* strains identified as the IAA producer may be developed as bioinoculant or biofertilizer to aid in the growth and development of the crop plants. These strains will not only help in plant growth & development but also will contribute in enhancing root/shoot growth and seedling vigor, cell elongation and cell division subsequently aiding in plant growth and development. This may help in attaining the ecofriendly sustainable and low-cost farming through the use of these strains identified in this study as having the IAA producing ability.

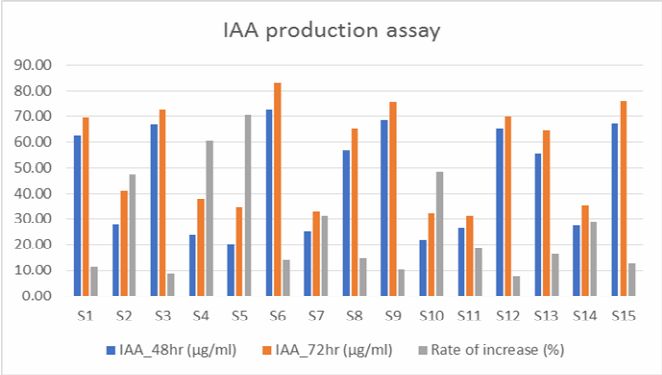


Fig. 1 : Concentration of IAA produced by the *Bradyrhizobium spp.*

Table 1 : ANOVA

Anova for IAA (µg_ml) produced after 48hr of incubation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Replication	2	0	0.2	0.183	0.834	
Treatment	14	18722	1337.3	1419.534	<2e-16	***
Residuals	28	26	0.9			

Anova for IAA (µg_ml) produced after 72hr of incubation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Replication	2	0	0.1	0.058	0.944	
Treatment	14	16425	1173.2	486.221	<2e-16	***
Residuals	28	68	2.4			

Table 2 : Concentration of IAA produced by bacterial strains at different time interval

Strains	OD_48 hr	IAA_48hr (µg/ml)	OD_72 hr	IAA_72hr (µg/ml)	Rate of increase (%)
S1	1.62	62.55d	1.81	69.67d	11.38
S2	0.69	27.70f	1.04	40.82f	47.37
S3	1.74	67.05b	1.89	72.67c	8.38
S4	0.58	23.59h	0.96	37.82g	60.36
S5	0.49	20.21i	0.87	34.45hi	70.43
S6	1.89	72.67a	2.16	82.78a	13.92
S7	0.62	25.08gh	0.83	32.95hij	31.37
S8	1.47	56.93e	1.69	65.17e	14.48
S9	1.78	68.55b	1.97	75.66b	10.39
S10	0.53	21.71i	0.81	32.20ij	48.32
S11	0.65	26.21fg	0.78	31.08j	18.58
S12	1.69	65.18c	1.82	70.04d	7.47
S13	1.43	55.43e	1.67	64.42e	16.22
S14	0.68	27.33f	0.89	35.20h	28.79
S15	1.75	67.42b	1.98	76.04b	12.78
LSD 5%		1.62		2.60	
CD		2.12		2.84	

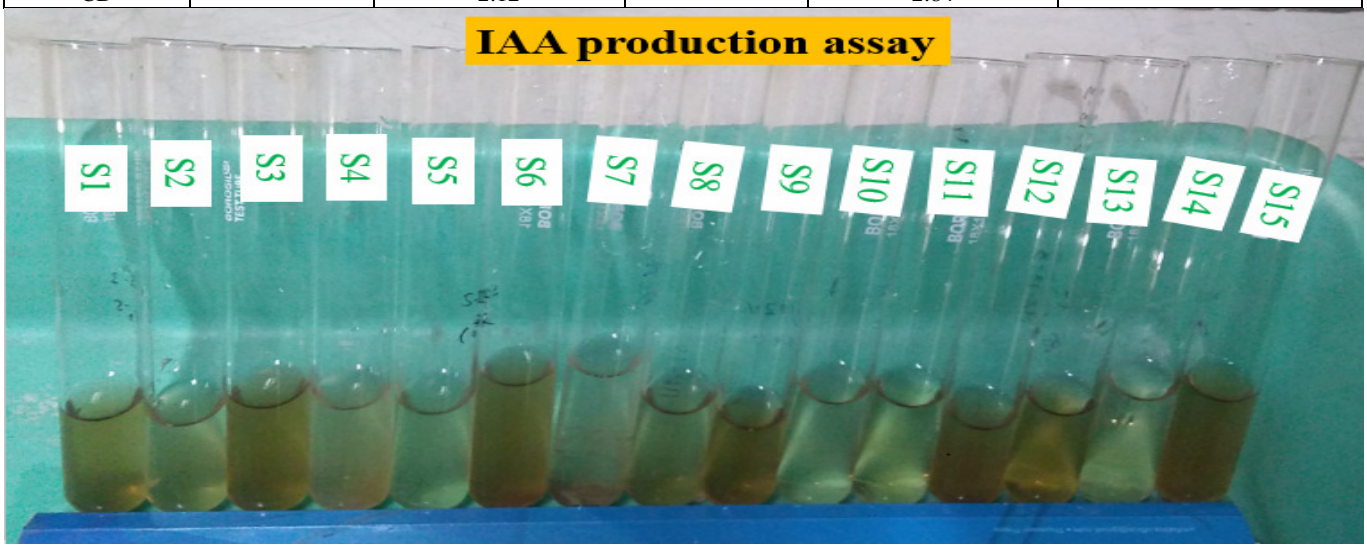


Plate 1 : Image showing the colour change due to IAA production by different bacterial strains

Table 1 : Concentration of IAA produced by Bradyrhizobium spp. after 48 hours and 72 hours

Strains	S1	S2	S3	S4	S6	S7	S8	S9	S10	S11	S12	S13	S14
OD_48 hr	0.17	1.21	1.32	1.36	1.29	0.86	0.74	1.16	0.61	0.85	0.68	1.18	0.89
Concentration of IAA (µg/ml)	8.22	47.19	51.31	52.81	50.19	34.08	29.58	45.32	24.71	33.70	27.33	46.07	35.20
OD_72 hr	0.2	1.69	1.61	1.74	1.82	1.17	0.84	1.58	0.93	0.98	0.89	1.49	1.13
Concentration of IAA (µg/ml)	9.35	65.17	62.18	67.05	70.04	45.69	33.33	61.05	36.70	38.57	35.20	57.68	44.19

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