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Study of production of indole acetic acid by soil and plant bacterial isolates on different media

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Abstract
PGPR have been reported to produce different phytohormones like auxins, gibberellins and cytokinins. IAA (indole-3-acetic acid) is the group of phytohormones and is considered the most important native auxin. Present study was carried out for quantitative estimation of IAA production ability of selected soil and plant isolates on nutrient agar broth. Isolates positive for P solubilization and those positive for K solubilization/mobilization were also evaluated on respective P and K solubilization media. Result showed, On nutrient agar media, the highest IAA production was recorded with D3 ($p<0.05$; 13.5 µg/ml broth) which was statistically at par with OS21 (12.94 µg/ml broth), MER4 (12.75 µg/ml broth) and D19 (12.335 µg/ml broth). On P solubilization media *i.e.* Pikovskaya broth out of 10 isolates, the highest IAA production was obtained with SEN3 (12.7 µg/ml broth). MER4 produced the highest amount of IAA (24.22 µg/ml broth) compared to others on Potassium solubilization media (Aleksandrov media).Out of 4 endophytic bacterial isolate from corn root, the highest IAA production was recorded with MER4 ($p<0.05$; 12.7 µg/ml broth).

Keywords: IAA, Nutrient broth, Aleksandrov media, PGPR, Pikovskaya broth

Introduction
In vitro production of auxins is a useful approach for selecting effective PGPR (Asghar *et al*., 2004) [3]. Auxins are the most abundant phytohormone secreted by most plant-associated bacteria. *Azospirillum* spp. are known for the production of indole-3- acetic acid, gibberellic acid and kinetin whereas *Azotobacter chroococcum* is identified to produce, indole-3- acetic acid. PGPR alter root growth in grasses by producing phytohormone. The effect of exogenous IAA in the plant can stimulate or inhibit growth and is often a function of hormones concentration available; in addition, the sensitivity of plant tissue changes according to hormones concentration (Persello-Cartieux *et al*. 2003) [13]. The highest concentration of IAA is produced by bacterial strain *P. fluorescens* and *Kocuria* varians. when applied in optimum concentrations, bacterial indole-3-acetic acid (IAA), synthesized by gram-positive and negative, photosynthetic, methylotrophic and cyanobacteria, is reported to stimulate root hair formation, at the same time increasing the length and the number of primary and lateral roots (Khalid *et al*. 2004). A *Streptomyces* isolate increased plant growth in wheat and produced indole acetic acid and auxin in presence of salt (Sachdev *et al*., 2009) [14]. Phytohormone-producing *Bacillus* sp. and *B. subtilis* have potential at field level to improve wheat productivity and may be helpful in formulation of an effective biofertilizer for wheat (Baghaee *et al*., 2014) [4]. The effect of inoculation of four IAA producing Pseudomonas isolates on α-amylase activity of durum wheat after six and 8 days of inoculation was significant, while after two and 4 days of inoculation was not meaningful and exogenous IAA displayed a concentration-dependent effect on seed germination attributes and α-amylase activity, consistent with the possibility that the inhibitory effect of bacterial inoculation on seed germination was in consequence of bacteria-produced IAA (Tabatabaei *et al*., 2016) [16]. In present investigation, we have examined the production of Indole Acetic Acid by soil and plant bacterial isolates on different media. IAA production ability tested for the isolates on complex media for (nutrient agar) showed difference in ability of different isolates for production of IAA. Further, with change in media, same isolates could produce different amount of indole acetic acid.

2. Materials and methods

Present investigation was carried out during rabi season of 2016-17 at Research Farm of ICAR-IISS, Bhopal to examined "Study of production of Indole Acetic Acid by soil and plant bacterial isolates on different media". The material used and methods adopted during the course of experimentation are described in brief as under following heads.

2.1 Sterilization of media and glasswares

All media were autoclaved at 15 psi (1.06 kg/cm^2) pressure for 20 minutes. Antibiotics, tryptophan stock were filter sterilized by using 0.22 µm disposable syringe filters. The glasswares were sterilized in hot air oven at 180^{0}C for 2 hours.

2.2 Indole Acetic Acid (IAA) Production

IAA production by the isolates was studied by the method of Hartmann (Hartmann *et al.,* 1983) [10]. Culture tubes containing 10 ml nutrient broth was prepared and autoclaved. Filter sterilized tryptophan solution (50µg/ml broth) was added to individual tubes. The tubes were inoculated with 200µl of different isolates, incubated at 28±2°C on an orbital shaker for 5 days. Uninoculated tubes served as negative control. Three replications for each treatment were maintained. The isolates showing P solubilization and those showing K solubilization were also tested for IAA production ability on Pikovskaya broth and Aleksandrov broth besides nutrient broth.

After incubation, IAA productions by the different isolates were determined. The culture broth was centrifuged at 5000 rpm for 15 minutes. The supernatant was used for the qualitative detection of IAA production by isolates. To one ml of the supernatant 4 ml of the reagent was added and mixed thoroughly. The tubes were incubated for 30 minutes to allow the colour to develop. Development of pink colour indicated IAA production by the isolate. Intensity of colour was read at 530 nm. Quantification of IAA production was done by preparing curve with standard indole acetic acid concentration against OD. Viable cell count was taken after serial dilution of culture and the result were expressed in terms of µg IAA/ml culture.

2.3 Statistical analysis

Statistical analyses were carried out through one-way analysis of variance (ANOVA) and the mean of treatments were compared according to Fisher's multiple comparison tests. Least significant difference (LSD) was calculated at $p<0.05$ using statistical package of SAS.

3. Results

3.1 Production of Indole Acetic Acid (IAA) by bacterial isolates

Many microbes have been reported to produce different phytohormones like auxins, gibberellins and cytokinins. IAA (indole-3-acetic acid) is the member of the group of phytohormones and is generally considered the most important native auxin. Quantitative estimation of IAA production ability of all the 32 isolates was carried out on nutrient agar broth. Isolates positive for P solubilization and those positive for K solubilization/mobilization were also evaluated on respective P and K solubilization media. On nutrient agar media, the highest IAA production was recorded with D3 ($p<0.05$; 13.5 µg/ml broth) which was statistically at par with OS21 (12.94 µg/ml broth), MER4 (12.75 µg/ml broth) and D19 (12.335 µg/ml broth). The lowest IAA production on Nutrient agar was recorded with isolate D4 ($p<0.05$; 0.76 µg/ml broth) (Table 6). On P solubilization media *i.e.* Pikovskaya broth out of 10 isolates, the highest IAA production was obtained with SEN3 (12.7 µg/ml broth) followed by JS2 (10.44 µg/ml broth) which was statistically at par with MER3 (9.88 µg/ml broth). Similarly, on Potassium solubilization media (Aleksandrov media), MER4 produced the highest amount of IAA (24.22 µg/ml broth) compared to others (table 8). Isolate JS4 produced 20.97 µg IAA/ml broth which was statistically at par with JS5 (18.14 µg IAA/ml broth) (Table 7). Among three different media used for IAA production assay, the production was recorded on Aleksandrov media supported maximum IAA production followed by nutrient agar and Pikovskaya broth was found to be the least supporter of IAA production. IAA production: 10.81 µg mL^{-1} (*Paenibacillus taichungensis*; M10).

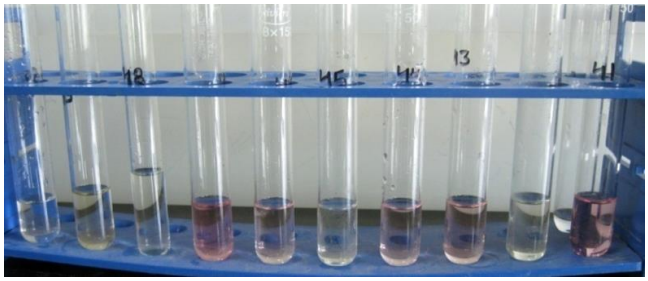

Fig 1: Indole acetic acid production ability of bacterial isolates

Table 1: Indole acetic acid production ability of bacterial isolates on nutrient broth media.

Isolate	IAA (µg/ml @10^7 cells/ml) mean	culture	IAA (µg/ml @10^7 cells/ml) mean
OS 2	4.44	JS 2	1.30
OS 10	1.21	JS 3	1.32
OS 14	5.51	JS 4	1.19
OS 16	2.82	JS 5	2.53
OS 18	4.57	MER 1	4.90
OS 21	12.94	MER 2	7.87
D 2	3.35	MER 3	6.72
D 3	13.50	MER 4	12.70
D 4	0.76	SEN 1	7.85
D 5	4.09	SEN 2	7.22
D 6	1.76	SEN 3	6.37
D 7	5.41	SEN 4	7.29
D 8	2.72	SRP 1	2.81
D 13	9.64	SRP 2	4.51
D 19	12.33	SRP 3	5.28
JS 1	1.87	SRP 4	2.33
LSD (0.05)	1.6		

Table 2: Indole acetic acid production ability of selected bacterial isolates on Pikovskaya broth.

Culture	IAA (µg/ml)
PSB media	
OS 10	0.29de
JS 1	0.59cde
JS 2	10.44b
JS 4	1.35c
JS 5	1.21cd
MER 3	9.88b
MER 4	0.41de
SEN 2	0.31de
SEN 3	12.70a
SEN 4	0.10e
LSD(0.05)	0.93

Table 3: Indole acetic acid production ability of selected bacterial isolates on Aleksandrov broth.

Culture	IAA (µg/ml)
KSB	
OS 10	8.56d
JS 1	11.15c
JS 4	20.97b
JS 5	18.14b
MER 3	24.22a
MER 4	8.98d
LSD (0.05)	2.5

4. Discussions

Indole acetic acid (IAA) is an important class of phyto-hormone known for improving plant growth by enhancing root and shoot growth of plants. IAA production ability tested for the isolates on complex media for (nutrient agar) showed difference in ability of different isolates for production of IAA. Further, with change in media, same isolates could produce different amount of indole acetic acid. We presume that some of the component in the media might act as precursor for synthesis of these phytohormones. Amount of the precursor and its availability to the microbes might influence the IAA production in different culture media. Idris *et al.*, (2007) [11] reported increase in the production of IAA in *Bacillus amyloliquefaciens* FZB42 with addition of tryptophan in culture media. Karnwal (2009) [12] tested *Fluorescent Pseudomonas* isolates for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan and found that for both strains, indole production increased with increases in tryptophan concentration. Isolates producing IAA have stimulatory effect on the plant growth. When the crop is inoculated with the isolates capable of IAA production significantly increases the plant growth by the N, P, K, Ca and Mg uptake of sweet potato cultivar (Farzana and Radizah, 2005) [9]. There is a significant increase in rooting and root dry matter of cuttings of eucalypts when grown on IAA producing rhizobacteria inoculated substrate. In present study four endophytic isolates were obtained from maize root produced indole acetic acid to the extent of 12.7µg/ml in nutrient broth media (MER4) and 24.227µg/ml (containing 10^7 cells/ml) in Potassium solubilizing media (MER3).

4. Conclusion

Conducted study concluded that, on nutrient agar media, the highest IAA production was recorded with D3 ($p<0.05$; 13.5 µg/ml broth) which was statistically at par with OS21 (12.94 µg/ml broth), MER4 (12.75 µg/ml broth) and D19 (12.335 µg/ml broth). On P solubilization media *i.e.* Pikovskaya broth out of 10 isolates, the highest IAA production was obtained with SEN3 (12.7 µg/ml broth). MER4 produced the highest amount of IAA (24.22 µg/ml broth) compared to others on Potassium solubilization media (Aleksandrov media).The highest IAA production was recorded with MER4 ($p<0.05$; 12.7 µg/ml broth).

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