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Short Communication

A SIMPLE AND RAPID PLATE ASSAY FOR THE SCREENING OF INDOLE-3-ACETIC ACID (IAA) PRODUCING MICROORGANISMS

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ABSTRACT : The indole-3-acetic acid (IAA) producing plant growth promoting rhizobacteria (PGPR) are routinely screened in the laboratory by colorimetric method in culture supernatant which is time consuming and expensive. We developed a novel plate assay for the detection of IAA in the bacterial strains. This method is rapid, cheap and accurate for IAA production in microorganisms.
Key words: indole-3-acetic acid; IAA; plate assay, PGPR, microorganisms

Indole-3-acetic acid (IAA) is a phytohormone, a type of best characterized auxins, which is essential for the growth and development of plants. It is most common and at the same time physiologically most active. It is known to stimulate both rapid (e.g., increases in cell elongation) and long-term (e.g., cell division and differentiation) responses in plants (Cleland, 1990; Hagen, 1990). The capacity to synthesize IAA is widespread among soil- and plant-associated bacteria. It has been estimated that 80% of bacteria isolated from the rhizosphere can produce the plant growth regulator IAA (Patten and Glick, 1996). Tryptophan (Trp) is generally considered to be the precursor of IAA. Several IAA biosynthetic pathways such as indole-3-acetamide pathway, indole-3-pyruvate pathway, tryptamine pathway, tryptophan side-chain oxidase pathway, indole-3-acetonitrile pathway and tryptophan-independent pathways have been reported in bacteria (Spaepen et. al., 2007), although, the best-characterized pathways in bacteria for the conversion of Trp to IAA are the indole-3-acetamide pathway and the indole-3-pyruvate (IPyA) pathway (Costacurta and Vanderleyden, 1995; Patten and Glick, 1996). Isolation of IAA producing microorganisms is done more frequently in the laboratory. The technique of Gordon and Weber (1951) is generally used for the isolation of IAA producing microorganisms. For this technique, each bacterial strain should be grown separately in culture media broth and colorimetric study is done in the supernatant which is time consuming. On the other hand, the plate assay can be done for many strains in a single plate in a less efforts. Therefore, a suitable plate assay was felt essential for a long time.

In the present study, we report here a novel, rapid and easy plate assay for the detection of IAA production in microorganisms. For the optimization and development of this technique, eight bacterial cultures namely *Klebsiella* sp. strain ECI-10A, *Agrobacterium* sp. strain AF-1D, *Pseudomonas* sp. strain AF-4B, *Klebsiella* sp. strain AF-4C, *Serratia* sp. strain AF-5A, *Pseudomonas* sp. strain PN-4D, *Agrobacterium* sp. strain BN-2A and *Klebsiella* sp. strain BN-4A were used which was isolated previously from the rhizosphere of rice plants from Indo-Nepal border region (U.P. shrivastava, 2009). These isolates were tested for IAA production ability. A novel, rapid and simple method for accurate screening of IAA producing microorganism were developed. They were tested for previously described IAA production screening method (Gordon and Weber, 1951) and compared with the newly developed plate assay for the same.

For the colorimetric technique proposed by Gordon and Weber (1951), the test isolates were grown in $JNFb^-$ liquid medium with or without tryptophan (100 µg/mL) at 30°C with shaking at 80 rpm for three days. The supernatant of 1.5 mL culture was recovered after centrifuged at 8000 rpm for 5 min. Thereafter, 1 mL supernatant was taken in a test tube and 2 mL IAA reagent (1 mL of 0.5 M $FeCl_3$ was mixed in 50 mL of 35% $HClO_4$) was added.

After incubation for 25 min at room temperature, the optical density of the samples was recorded at 530 nm in the respect of blank medium as negative control. The amount of IAA was calculated with standard of pure IAA prepared separately.

For newly developed plate assay, JNFb$^-$ agar plate containing 100 µg/mL of tryptophan was prepared. After proper solidification, cavities of approx. 1-2 cm diameter and 0.5 cm depth were made by means of sterile cork borer. 200 µL of overnight grown culture was poured in each cavity and incubated at 30^{o}C in a BOD incubator. After overnight growth, the cultures were removed gently with tissue paper from the cavities and 200 µL of IAA reagent was added in the cavity. After addition of reagent, pink halo zone appeared around cavity which was measured including the whole cavity. The diameter of the cavity and the total halozone diameter of all strains were measured at the time of test and thereafter, IAA production index of each strain was calculated by the formula and strategy shown in Fig. 1 and the data is shown in table 1. The IAA production index was correlated with the quantity measured by the method of Gorden and Weber, 1951. It is evident with the data shown in table-1 that the IAA production index of almost all stains showed correlation with the amount of IAA estimated by the method of Gordon and Weber, 1951. It was observed that the rate of diffusion of IAA on the agar plate was directly proportional to the diffusion potential of the IAA which represented their concentration; therefore, the diameter of hazozone may be represented for the amount of IAA produced. Higher the amount of IAA showed larger the size of halozone diameter in a definite time. Therefore, we report here that comparative tentative amount of IAA production can be correlated in a single agar plate by this method. Though, the calculation of IAA production index in this pattern is not a general trend, but it was used for the first time to check the authenticity of the cavity diffusion technique. Furthermore, the IAA production index may be calculated as follows in future work.

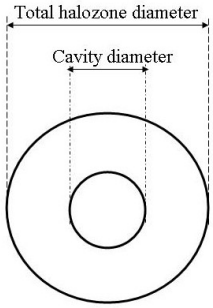
$$\text{IAA Production Index} = \frac{(\text{Total halozone diameter} - \text{Cavity diameter})}{\text{Cavity diameter}},$$

Except the above mentioned work, test was also made by spot inoculation by the diluted inoculum on solid agar plate and after appearance of colonies IAA reagent was over layered, such type of pink coloured halozones also appeared around colonies in IAA producers whereas no pink halozone around IAA production lacking strains.

Table 1: Quantitative estimation of IAA, Halo-zone diameter for IAA and IAA production index after three of bacterial growth

Bacterial strain	IAA Production (µg/mg dry weight)	Halo zone diameter (mm)	IAA production Index
Klebsiella sp. strain ECI-10A	5.35	7	0.76
Agrobacterium sp. strain AF-1D	6.57	9	0.73
Pseudomonas sp. strain AF-4B	2.3	3	0.77
Klebsiella sp. strain AF-4C	5.7	8	0.71
Serratia sp. strain AF-5A	6.69	8	0.84
Pseudomonas sp. strain PN-4D	1.92	3	0.64
Agrobacterium sp. strain BN-2A	16.36	21	0.78
Klebsiella sp. strain BN-4A	4.67	6	0.78

Quantitative estimation and Halozone diameter data are based on average of three readings performed separately in identical conditions.
IAA production index is determined by following formula:
Halozone diameter = Total halozone diameter – Cavity diameter
IAA production Index = Amount of IAA (µg/mg dry weight)/ Halozone diameter (mm)



$$\text{Halozone diameter} = \text{Total halozone diameter - Cavity diameter}$$

$$\text{IAA Production Index} = \frac{\text{Amount of IAA (mg/mg dry weight)}}{\text{Halozone diameter}}$$

Fig.1: Diagrammatic representation of diameters of total halozone and the cavity used for the calculation of IAA production index.

The detection of IAA production in liquid culture by colorimetric method is in use since more than five decades, but rapid method of detection in large number of isolates at a time is lacking. To solve this problem attempts was made and satisfactory result was obtained, in due course a novel plate assay for the detection of IAA has been developed. In the agar plate assay, the principle of colour development with IAA reagent (1: 50 ratio of 0.5 M $FeCl_3$ and 35% $HClO_4$) was kept unchanged with a presumption that IAA produced by the culture shall diffuse into the solid agar medium and subsequently react with IAA reagent. It was observed that all the eight bacterial strains showed pink zone around the cavity, although the diameter of pink zone varied most probably due to the amount of IAA produced by individual isolate (Fig. 2B). Appearance of pink zone was absent in IAA non-producers (Fig. 2A). To check the reproducibility of this test, it was repeated several times and identical result was obtained when the all conditions was remain constant. Altogether plate assay seems rapid and accurate method for the detection of IAA production.

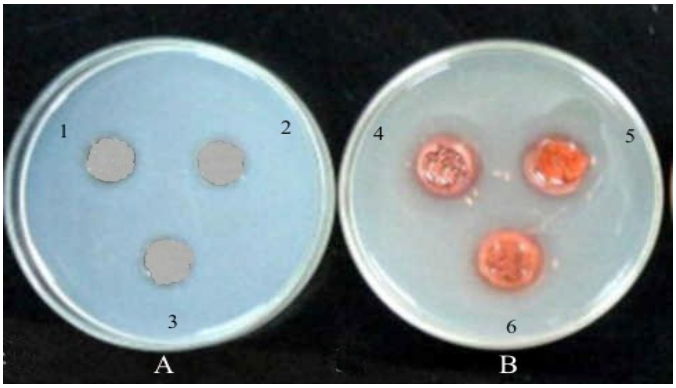


Fig. 2: Photograph showing IAA production in plate assay. **A** - No IAA production in three isolates (1,2 and 3), and **B**- IAA production by three isolates (4, 5 and 6).

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