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Extraction and Identification of Indole-3-Acetic Acid Synthesized by Rhizospheric Microorganism

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Abstract

IAA is a key regulator of plant growth and development. The growth regulation is mainly dependent of the change of free Indole 3 acetic acid levels in the target tissues, quantification of indole-3-acetic acid (IAA), the most abundant natural auxin, is indispensable in the study of auxin action. Currently; spectrophotometry techniques like HPLC are technically the best methods to measure Indole 3 acetic acid, due of high sensitivity and specificity. However, its high cost for setting and maintenance makes it difficult for daily use in ordinary laboratory. Therefore, establishment of a standard method to quantify IAA based on different spectrophotometric techniques, ensure the specificity and concentration of auxins like indole 3 acitic acid.

Keywords: *IAA; HPLC; Auxin; Spectrophotometry.*

1. Introduction

World's population is still growing; food production needs to be increased. Agriculture is backbone of food with high yield of legumes and cereals. Soil needs a solid input for a higher output. Soil fertility must be increased with the productivity of crops.

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Plant growth promoting bacteria have influenced plant growth in different modes as the production of plant growth regulators like auxins, cytokinin, gebrallinetc.The discovery of plant growth regulators during the nineteenth century was the outcome of experiments on phototropism and geotropism[1]. These plant growth promoters increase the productivity. The growth of plant with the soil microbes that are surrounding the rhizosphere region may be of beneficial, neutral or harmful and the bacteria thus dominate positive aspect are called rhizobacteria.[2] Indole-3-acetic acid (IAA) is the most abundant natural auxin that plays diverse roles in plant growth and development[3].The activity of auxin can be regulated by changing the endogenous level of free IAA or the sensitivities of target tissues[4]. Although the latter means that the differential responses to the given concentration of auxin, the exact mode of sensitivity change is achieved by modifying the dose-response curve to IAA in the target tissue[5] Therefore, detection and quantification of IAA in a living tissue is indispensable to study the plant growth regulation. Quantification is also a central part of auxin biosynthesis research[6].

2. Material and Methods

2.1 Isolation and identification of Pseudomonas fluorescence

Rhizosphere soil samples were isolated by serial dilution method using sterile nutrient agar. The isolates were further grown in King's B medium. The strain that showed fluorescence was selected and purified for further study. Identification of the isolates was carried out using colony morphology, Gram reaction and motility. Biochemical characterization was done following the standard method described in Bergey's manual of systematic Bacteriology.

2.2 Media for production of IAA

The isolated *Pseudomonas fluorescence* strain was used for IAA production. The production medium containing (w/v): L - tryptophan - 0.5%, NaNO3 - 0.2%, K2HPO4 - 0.1%, MgSO4.7H2 O - 0.01%, CaCO3 - 0.2% and Glucose - 1% was inoculated with the culture and incubated at 280C for 72 hrs in a rotary shaker at 120 rpm. Bacterial growth was determined using spectrophotometer taking optical density at 540 nm at every 12 hours intervals. Bacterial cells were removed by centrifugation at 4,000 rpm for 20 min and the cell free supernatant was used for determining the concentration of Indole acetic acid[7].

2.3Optimization of cultural and nutritional conditions for Indole acetic acid production

The following parameters were studied to determine their influence on IAA production. The incubation period was studied by inoculating into 100 ml L-tryptophan supplemented production medium and incubated for 72 hrs at 280C in a rotary shaker at 120 rpm. Samples were withdrawn every 24 hrs and growth.

2.4 Purification and Detection of Indole acetic acid

Purification of IAA from the sample was done by column chromatography using dry silica gel as absorbent and the solvent used was ethyl acetate and hexane (20:80 v/v). The sample was loaded to the silica gel column and the fractions were collected.

2.5 Thin layer chromatography

The fractions (10 - 20 micro litter) and standard IAA were placed on TLC plates (silica gel G f254 thickness 0.25mm). TLC was run by using solvent system benzene: n-butanol: acetic acid in 70:25:5 proportion and spots were detected by spraying the plates using Salkowaski reagent. Rf value of the standard and IAA produced by the isolate was calculated.

2.6Thin Layer Chromatographic Analysis

Partial purification of Indole Acetic Acid from crude extract was done by using silica gel column chromatography and fractions were collected with solvent system ethyl acetate and hexane (20:80 v/v). Each fraction was tested in thin layer chromatography and then developed with Salkowski reagent. Chromatogram of culture showed a pink spot of purified indole acetic acid at the Rf value (0.62) almost same to standard IAA (0.67). Thin layer chromatography findings are in agreement with reports in [8,9&10].

3.0 Results

Among the bacterial isolates which were grown on Jensen's medium that produce pink color was biochemically identified as Azotobacter using Bergey's manual of systematic bacteriology. This also confirms the pilot scale identification of the indole -3- acetic acid produced by Azotobacter. This technique may have future significance on the production of biobased fertilizer.

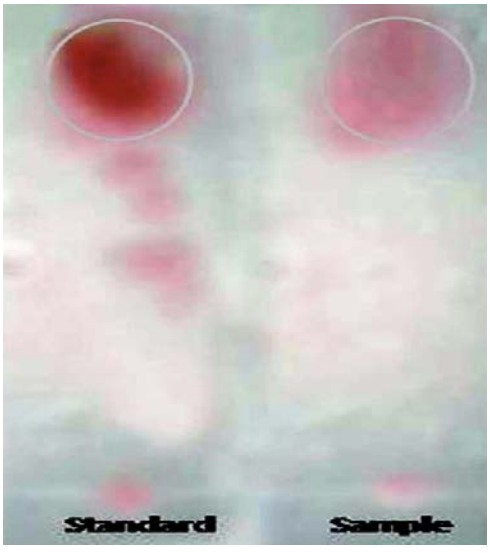

Fig. **1 Showing results of Thin Layer Chromatography**

4. Conclusion

Azotobacter has the capability to produce indole-3-acetic acid, which is an essential auxin for plant growth. The effect of IAA on plants is being explored through ongoing plant inoculation studies. The use of this strain as a producer of bio-based fertilizer will also help farmers to utilize the capability of this microorganism. The use of this bio-based fertilizer shows 20% increment in yield when use on the pilot scale. Effective extraction of such Plant Growth regulators is a real hope to maximize crop production in order to meet the food demands of the growing population. IAA like other growth regulators is capable of effectively growing multiple crops and has no side effects on any. This reduces the dependence on chemically based fertilizers, which are important to insure the environment protection as well as health.

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