**International Journal of Universal Print**
ISSN: 2454-7263 ID: ACTRA 2018 064 Published Mar. 2018
Volume No. 04, Issue No.07, Copyright © Universal Print
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Study of IAA production by *Rhizobium* …

# STUDY OF INDOLE ACETIC ACID PRODUCTION BY *RHIZOBIUM LEGUMINOSARUM* UNDER DIFFERENT SET OF CONDITIONS

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***ABSTRACT***

*Plant growth promoting rhizobacteria (PGPR) are group of bacteria that actively colonize plant roots and increase plant growth and yield. Direct methods of increasing plant growth are through production of phytohormones, such as auxin, cytokinin and gibberellin. These studies indicate that higher the plant growth promoting activities of rhizobacteria, higher is the chance of increased plant growth under different conditions. A number of different bacteria promote plant growth, including Azotobacter sp., Azospirillum sp., Pseudomonas sp., Bacillus sp. and Acetobacter sp.. Plant growth promoting bacteria are important in managing plant growth because of their effects on soil conditions, nutrient availability, growth and yields. Therefore, the aim of this study was to check IAA production by Rhizobial isolates under different set of conditions.*
**Key Words: IAA, PGPR, Phytoharmone, Rhizobium, Rhizobacteria.**

## 1. INTRODUCTION

Microorganisms synthesize auxins and gibberellins like compounds and these compounds increase the rate of seed germination and development of root hair that aid plant growth .

The action and interaction of some growth regulators like auxins regulate most of the physiological activities and growth in plants. Naturally occurring substances with indole nucleus possessing growth-promoting activity are referred to as auxins. Chemically it is Indole acetic acid. The ability to synthesize phytohormone is widely distributed among plant associated bacteria. 80% of the bacteria isolated from plant rhizosphere are to produce IAA [13] According to Halda-Alija L.2003 [8], up to 74% of rhizobacteria identified and tested produce IAA.

Auxins generally occur as complexes, usually bound to an amino acid

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or sugar. Over six different precursor molecules for auxins have been reported. Many workers reported that the amino acid, tryptophan figures prominently in the auxins formation .Auxins are employed to induce rooting, callus formation, flowering, parthenocarpy. They can also prevent abscission of leaves, flowers and fruits. Thus their application potential is enormous.

Bacteria in the Genus *Rhizobium* reduce atmospheric nitrogen to ammonia when they are located in the root nodules of leguminous plants. The formation of these root nodules is dependent on a complex series of interactions between the bacterium and the plant and requires growth and differentiation on the part of both partners. The plant growth regulator indole acetic acid (IAA) has long been postulated to play a role in one or more aspects of nodule growth and development and the detection of increased levels of IAA in nodule tissue supports this hypothesis [2]. The associative nitrogen-fixing bacteria tested produced IAA, especially with tryptophan as a precursor [10].

The phytohormone auxins plays a central role in plant growth and development as a regulator of numerous biological processes, from cell division, elongation and differentiation to tropic responses, fruit development and senescence [9]. Not only plants but also microorganisms can synthesize auxins and cytokinins. The role of phytohormone biosynthesis by microorganisms is not fully elucidated. But it was indicated that there might exist a symbiotic association between plants and microorganisms.

Hence the present study was undertaken to isolate organisms from nodules of leguminous plants and its rhizosphere and to study IAA production under laboratory condition.

## 2. MATERIAL AND METHODS

### 2.1 ISOLATION OF *RHIZOBIUM LEGUMINOSARUM*

Leguminous plants selected for the present study was *Arachis hypogaea* L. For isolation of Rhizobia from root nodules of leguminous plants standard method as described by Dubey and Maheshwari [6]; Deshmukh [1] was used by using Yeast extract mannitol agar( YEMA) with congo red Typical rhizobial colonies were opaque, white and mucoid. Isolates were identified as per Bergey'smanual of Systematic bacteriology [5].

### 2.2 SCREENING FOR IAA PRODUCTION:

The isolate was screened for IAA production by using Salkowaski reagent prepared as 2% 0.5 M FeCl**3** in 35% perchloric acid.Reaction was allowed to proceed until adequate colour developed. All regent incubations were carried out at room temperature. IAA produced was measured spectrophotometrically at 530 nm. TLC was carried out to confirm IAA production by the isolate [4, 7-11].

### 2. 3 IAA PRODUCTION IN DIFFERENT MEDIA

To select the best medium for production of IAA, different media are inoculated with 1% inoculum of $O.D._{600}$ 1.0 and incubated at RT for 24 hrs to 72 hrs. Media selected contain different nitrogen sources such as soybean casein digest, aspargine, and tryptophan. IAA production is checked after 24, 48 and 72 hrs by Gordon and Weber's method.

The media selected are:

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- Yeast extract mannitol broth with soybean casein digest as a source of tryptophan (YEMBSCD)
- Medium supplanted with mannitol 1%, SDS 1microgram/ml, L-Aspargine 0.02% w/v, biotin 1microgram/ml and tryptophan 2.5 mg/ml (A.C. Ghosh, Basu 2002) (TASB)
- Medium supplanted with mannitol 1%, SDS 1microgram/ml, L-Aspargine 0.02% w/v, biotin 1microgram/ml and tryptophan 2.5 mg/ml glucose 1%, $ZnSO_4$ 0.5 microgram/ml (Bhattacharya, Pati B.R.2000) (TAGZ)
- YEMB with 2.5 mg/ml of tryptophan(YEMB)
- YEMB without tryptophan

After incubation IAA produced is detected by Gordon and Weber's method

**2.4 EFFECT OF TRYPTOPHAN CONCENTRATION:**

Effect of tryptophan on IAA production was studied by using YEMB amended with 1- 5mg/ml, as well as 5mM were inoculated with the selected isolates as 1% inoculum of O.D.600 1.0 and incubated at 28°C for 24 hrs. IAA produced was measured spectrophotometrically at 530 nm.

**2.5 EFFECT OF CARBON SOURCES ON IAA PRODUCTION:**

Ability of isolates to produce IAA in presence of different carbon sources was studied. For this mannitol in YEMB is replaced by 1% glucose, sucrose, lactose, arabinose , xylose in presence of 2.5 mg/ml of tryptophan. IAA production was studied by using Salkowaski reagent after24, 48 and 72 hrs.

**2.6 EFFECT OF PH ON IAA PRODUCTION:**

To study the extent of IAA produced by the isolate at different pH, YEMB with 2.5mg/ml of tryptophan is adjusted to different pH as 5, 6, 7, 8 and 9. Media were inoculated
with 1% inoculum of O.D.600 1.0 and incubated at 28°C for 24 hrs. IAA production was studied by using Salkowaski reagent after 24hrs.

**3. RESULTS AND DISCUSSION**

**3.1 ISOLATION AND IDENTIFICATION OF RHIZOBIUM:**

The isolate was identified as Rhizobium according to the typical colonies produced on YEMA with congo red. The colonies were white colored, mucoid, and like a drop of water. The isolate was further confirmed by biochemical characters [12].

**3.2 SCREENING OF IAA PRODUCTION:**

The isolate was screened for IAA production by using Salkowaski reagent which gives typical red colour confirming IAA production. Confirmation of IAA was carried out by TLC separation. Rf value of standard IAA and that produced by the isolate are 0.78 confirming the production of IAA by the isolate.

**3. 3 IAA PRODUCTION IN DIFFERENT MEDIA**

For screening of the best suitable medium for optimum IAA production, the isolate was inoculated in various media viz

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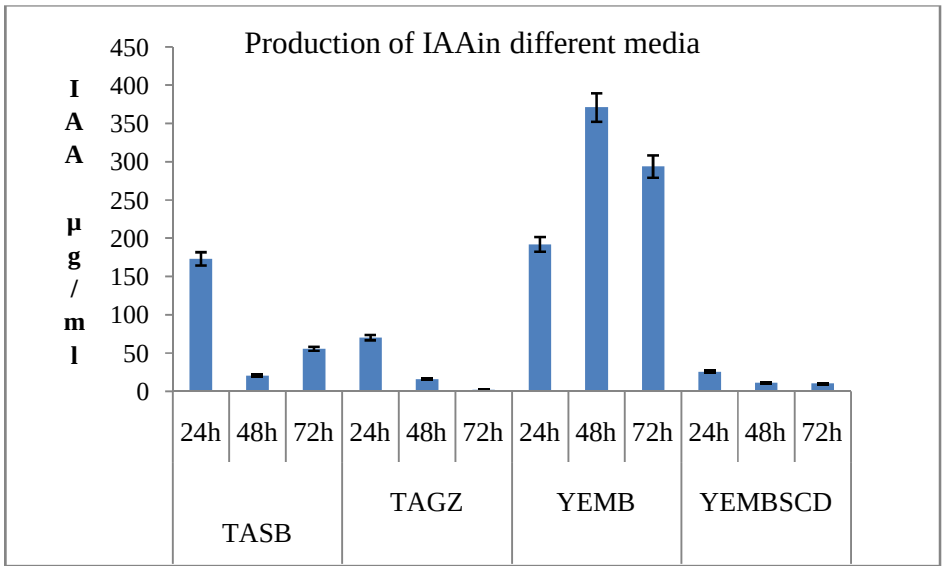
TASB, TAGZ, YEMB and YEMBSCD. YEMB without tryptophan did not show production of IAA indicating tryptophan requirement of the isolate for IAA production. Maximum IAA production was reported in YEMB with tryptophan (Fig 1) . Hence the same medium is used for further study.

**Fig 1 Production of IAA in different media**




### 3.4 EFFECT OF TRYPTOPHAN CONCENTRATION

The isolate was inoculated in YEMB amended with various concentrations of tryptophan from 1mg/ml to 5mg/ml. As the concentration of tryptophan in the medium increases, the amount of IAA produced increases (Fig 2). The isolate showed maximum production in 72 hrs.

**Fig 2 Effect of tryptophan concentration on IAA production**

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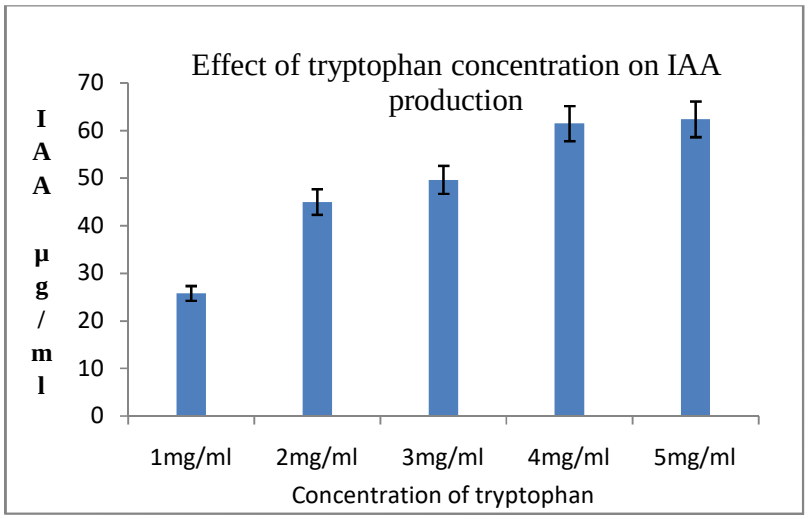
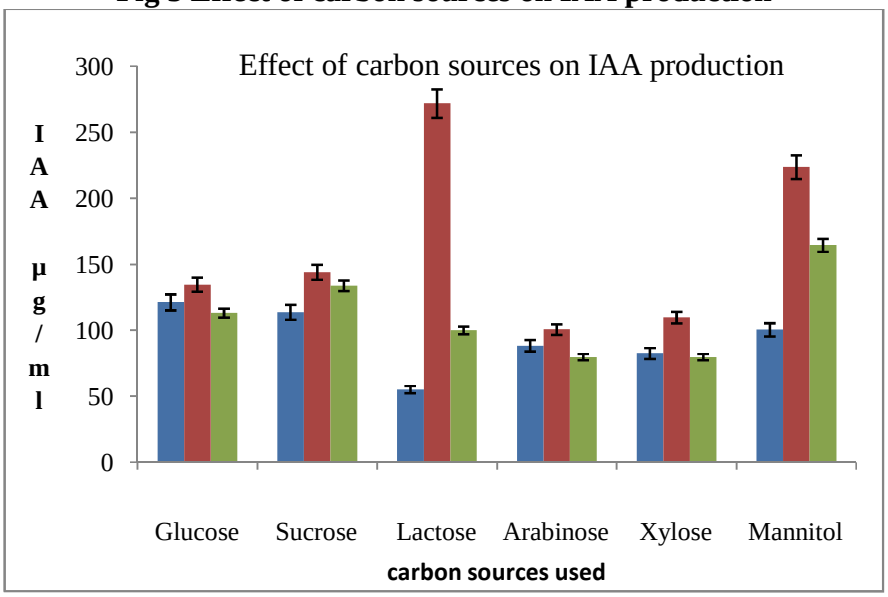



### 3.5 EFFECT OF CARBON SOURCES ON IAA PRODUCTION

The isolate responded in varied manner to different carbon sources. *Rhizobium leguminosarum* showed maximum IAA production in presence of glucose, lactose and mannitol in 48 hrs. In 24 hrs *Rhizobium leguminosarum* gave maximum production of IAA in presence of glucose (Fig 3). Basu & Ghosh [3] have reported that glucose and KNO3 are the best carbon and nitrogen sources for IAA production by Rhizobium spp.

**Fig 3 Effect of carbon sources on IAA production**



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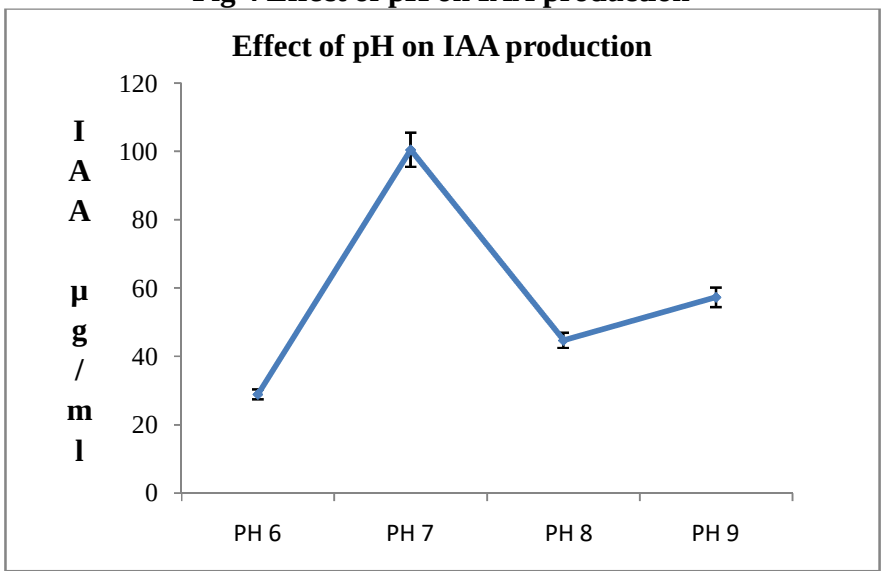
### 3.6 EFFECT OF pH ON IAA PRODUCTION

*Rhizobium leguminosarum* did not show IAA production at PH 5 and 6. The isolate showed maximum IAA production at PH 7. The results indicate optimum PH for IAA production is 7 (Fig 4).

**Fig 4 Effect of pH on IAA production**




It can be concluded that the IAA produced by the isolate can be used as sprays for plant growth promotion. Co- inoculation of rhizobia with other plant growth promoting bacteria can be done for growth promotion.

### 4. ACKNOWLEDGEMENT

The author is thankful to the Dr.Babasaheb Ambedkar Marathawada University, Aurangabad for funding this project and is also thankful to the Principal, Maulana Azad College, Aurangabad for providing the necessary infrastructure to carry this work.

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