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**RESEARCH ARTICLE**

# EVALUATION OF THE PERFORMANCE OF INDIGENOUS AZOSPIRILLUM OF THE PLANT GROWTH UNDER POT CULTURE FROM MADURAI DISTRICT

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**ABSTRACT**

*Azospirillum* is the foremost common plant growth-promoting rhizobacteria (PGPR) that are generally associated with fibrous root containing plants viz., rice, wheat and Ragi. *Azospirillum* is a nitrogen fixing bacterium and secretes plant growth hormone like IAA, Gibberellin which helps in plant root growth. The performance of *Azospirillum* will be varying for different location due to its adaptability to the particular environment. In this experiment, the indigenous strains were isolated from rice. The isolates were purified, characterized, screened and mass multiplied on N free malic acid (Dobreiner, et.al, 1976). The screened isolates were subjected to pot culture condition. The effect of the isolates on plant root length, shoot length, dry weight, no of main and lateral roots were examined at 10, 20 and 30 DAS. Among the isolates isolate $AI_7$ gave better performance towards root length (18 cm), shoot length ( 35 cm), root volume (0.19 cc), number of main root ( 16 ) and number of lateral root (15.64) and dry weight (0.64g/plant).



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# INTRODUCTION

*Azospirillum* is a microbe belonging to the class of Plant Growth Promoting Rhizobacteria (PGPR).*Azospirillum*is a smaller scale aerophilic, gram negative, rod shaped, plant growth promoting bacteria, which grows well in Nitrogen free semi-solid malate medium.Azospirillum species are found in the rhizosphere of most important crop plants and are able to fix N under the conditions of microaerophilic. The isolation and use of Azospirillum was initiated from 1970 (Steenhoudt and Vanderleyden, 2000). Azospirillum consists of 10 species, including

*Azospirillumbrasilense, Azospirillumamazonense, Azospirillumirakense, Azospirillumlipoferum, Azospirillumlargimobile, Azospirillumhalopraeferens, Azospirillumoryzae, Azospirillumcanadensis, Azospirillumdoeberereinerae, and Azospirillummelinis*

(Tarrand *et al*., 1978; Magalhães *et al*., 1983; Dekhil *et al*., 1997; Peng *et al*., 2006; Mehnaz *et al*., 2007; Saharan and Nehra, 2011).The first individuals who indicated the production of ABA by Azospirillum in defined culture were Kolb and Martin (1985). Here, *Azospirillum* strain was isolated from AC & RI, Madurai and studied for the plant growth under pot culture conditions. The biochemical tests and estimation of IAA production were conducted to examine further about *Azospirillum*. This article briefly explains about the foresaid experiments in a well-organized manner.

# MATERIALS AND METHODS

In order to evaluate the growth performance of ***Azospirillum***, initially the microbe has to be isolated and then purified. After that broth cultures are developed. Then finally, mass production is started using Fermentors.

**ISOLATION:** To begin with isolation, the medium used is N-free Malic Acid semi solid medium (Malic Acid (5g), Potassium Hydroxide (4g), Dipotassium Hydrogen Orthophosphate (0.5g), Magnesium Sulphate (0.2g), Sodium Chloride (0.1g), Calcium Chloride (0.2g), Agar (1.75g), Iron EDTA (4 ml), trace element solution (2ml), Bromothymol blue (0.5%)) .Fresh roots of graminaceous plants like rice are collected and are inoculated into the fresh N-free Malic acid semi solid medium and kept for incubation under 30 ± 1 ºC. It nearly takes a week (5-7 days) for proper growth of the microbe. By this way the colonies are isolated and their growth is simultaneously observed.

**PURIFICATION:** Purification process is followed by isolation. This process helps to isolate a pure individual cell from isolated colonies. Slants are used to check the purity of ***Azospirillum***. Slants are prepared using N-free Malic acid medium and the isolated ***Azospirillum*** colonies are streaked in the slants. These are incubated under 30 ± 1 ºC for 5-7 days. Purification process is followed by

**BIOCHEMICAL CHARACTERIZATION:** Biochemical Characterization utilizing different biochemical tests. Carbon source utilization test, Glucose production from acid test and Biotin Requirement test are performed. Characterization was also performed using Gram Staining.

**IAA ESTIMATION**

*Azospirillum* microbe produces visible quantities of Indole Acetic Acid (IAA) and this is estimated using the foresaid procedure. It requires the preparation of Glucose yeast extract peptone broth (250 ml) and L-Tryptophan solution (100 ppm). 100 ppm of L-Tryptophan is prepared and added to the glucose yeast extract peptone broth. Finally the culture is inoculated into this broth and incubated under dark for 7-10 days. After this, the extraction procedure gets started and initially 25 ml broth culture is centrifuged at 10,000 ppm for 5-10 mins to palletize the cells. The cell free extract is collected using decantation and acidified using 0.1N HCl to pH 2.8. This extract is mixed with Diethyl etherin a separating funnel and kept in refrigerator at 4°C. After 4 hrs, the solvent phase is withdrawn and evaporated to dryness in a beaker. The residue is then dissolved in 2 ml methanol for quantitative estimation of Indole Acetic Acid. Here, the sample is mixed with 4ml Salpher's reagent and incubated under dark for 1 hr. Then, the color intensity is read usingspectrophotometer at 535 nm and the quantity of IAA produced is calculated using the standard curve.

**MASS PRODUCTION:** Finally, the broth culture of 3litres capacity was developed with the help of starter culture. After the complete growth of ***Azospirillum*** in broth, mass production gets started in fermenters. The evaluation of this indigenous ***Azospirillum*** was performed using Pot Cultures. Rice seeds were soaked overnight and were sown in pots. As soon as the seedlings start to emerge the ***Azospirillum*** was inoculated into these pots. Two controls and three replications/treatments were maintained. The root lengths of the ***Azospirillum*** treated seedlings as well as control seedlings were compared and their growth was recorded.

# RESULTS AND DISCUSSION

The results of the above foresaid experiments like isolation, purification, Biochemical characterization, IAA production are categorized below,

**Fig. 1. Isolated plate**

**ISOLATION AND PURIFICATION**

Isolation of *Azospirillum* was made from rhizoplane of Paddy root samples obtained from Agricultural College and Research Institute Campus, Madurai district. Nearly, six*Azospirillum* isolates were obtained byadopting enrichment culturetechnique.

**Fig. 2. Azospirillum Slants**

Thecolony morphology of isolates on N-free malate medium wassmall to medium, pale white dense, spindle and transparentpale shiny white in color. All the isolates formed subsurfacepellicles in NFBTB medium and turned olive green color ofBromoThymol Blue (BTB) to brilliant blue.Formation of pellicle in NFBTB indicates the isolates seem tobe *Azospirillum spp*. The pellicleformation may be considered as one of the criteria foridentification.The morphological characteristics of the isolates in thepresent study were appeared similar to the description of*Azospirillum spp.* given by Krieg and Dobereiner (1984) and Tarrand *et al*. (1978).Slants are used to check the purity of *Azospirillum*. The results show that the streaked slants visually show the color change from olive green to blue.

**BIOCHEMICAL CHARACTERIZATION**

All the isolates were tested for biochemical characteristics. Undercarbon source utilization test, thetest tubes showed the appearance of turbidity whichindicated utilization of glucose. Biotin requirement test shows Biotin free medium favored the growth of *Azospirillumbrasilense*, *Azospirillumlipoferum* required biotin for growth.The change in color of BTBfrom green to yellow was recorded in the Glucoseproduction from acid test.

**IAA PRODUCTION:** This study began with the isolation of bacteria type strain *Azospirillum sp.* and developed the investigation to a screening of their ability in IAA production. This screening conducted a selection of only bacteria that was capable of the production of IAA with its content of over 28 mg mL-1 for sequencing.Various factors that influence *Azospirillum sp.*in biosynthesizing IAA such as temperature, pH, nitrogen presence and concentration of tryptophan in the culture medium were examined. The results indicated that the culture conditions were suitable for IAA biosynthesis at pH 6.5, 30 °C, culture media with nitrogen, and 0.1% tryptophan.

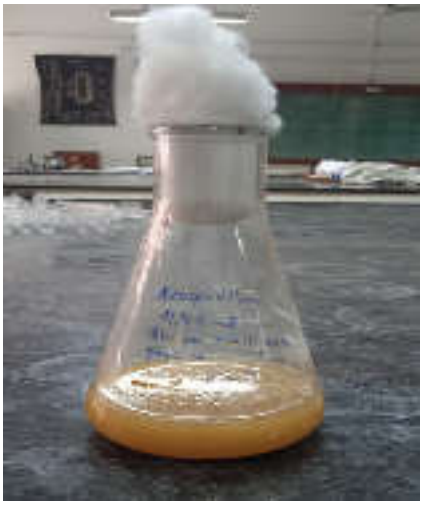

**Fig. 3. Glucose peptone broth**

**Fig.4 Centrifuged broth containing Azospirillum**

**MASS PRODUCTION:** The isolated strains were used for mass inoculums production. The isolates were stored in the refrigerator under lowtemperature. These strains are called starterculture. These cultures were then transferredinto 100 mL, 250 mL conical flask containing N- free malic acid semisolid medium incubated into for28±20ºC for one week. Then the motherinoculums were transferred into 500mL,1000mL conical flask containing N- free Malic acid liquidbroth (without agar). These flasks wereplaced in the shaker using occasional shakingfor 7 days for proper aeration and agitation.After 7 days, the liquid culturecontaining ***Azospirillum***were mixed withcarrier material. Lignite powder was used ascarrier. The carrier material 4 kg was mixedwith 1 liter of culture broth with 2% CaCO3was mixed and then the mixture is placed in apolythene sheet and covered with polythenesheet for 24 hrs for curing. Then the mixturewas pocketed into polythene bags and sealedit. The polythene bags were stored into thestore room at 28±20ºC. These culture pocketswere used for field application.

**Fig. 3. Glucose peptone broth**

**Fig .6. Broth after incubation and growth**

**POT CULTURE EVALUATION:** Pot culture is a method of growing treatments of biofertilizer treated seedlings and observing their root as well as shoot length. This evaluation is performed using rice seedlings.

**Fig.7. Azospirillium inoculated Rice seedlings under Pot Culture Conditions**

**Fig.8 Biometric Observation of Azospirillum treated Rice seedlings**

Rice seeds (ADT 45 Variety) were soaked overnight and were sown in pots. As soon as the seedlings start to emerge the ***Azospirillum*** was inoculated into these pots. Two controls and three replications/treatments were maintained. Under these two controls, no azospirillum was inoculated. In replications, T1 was inoculated with 50 ml of***Azospirillum***, T2 with 100 ml and T3 with 150 ml. After few days of their growth, the root as well as shoot lengths were measured which is given below in Table.1

**Table 1. Root and Shoot Lengths of Pot Culture Plants**

| Sample | Root length (cm) | Shoot length (cm) | Root volume (cc) | No. of Main roots | No .of lateral roots |
|---|---|---|---|---|---|
| **INITIALLY BIOMETRIC OBSERVATION ( 10 DAS)** | | | | | |
| **Control -1** | 9.5 cm | 17 cm | 0.10 | 5 | 4.56 |
| **Control -2** | 4.5 cm | 13 cm | 0.20 | 7 | 4.43 |
| **T1 ( 50 ml)** | 8.5 cm | 21.5 cm | 0.10 | 10 | 5.10 |
| **T2 ( 100 ml)** | 4.8 cm | 13 cm | 0.38 | 9 | 5.02 |
| **T3 (150 ml)** | 3.5 cm | 26 cm | 0.28 | 10 | 4.08 |
| **AFTER 20 DAYS OF OBSERVATION** | | | | | |
| **Control -1** | 11 cm | 25 cm | 0.15 | 7 | 7.45 |
| **Control -2** | 7 cm | 27 cm | 0.10 | 8 | 7.50 |
| **T1 ( 50 ml)** | 9 cm | 24 cm | 0.20 | 9 | 12.75 |
| **T2 ( 100 ml)** | 6.5 cm | 23 cm | 0.25 | 11 | 12.45 |
| **T3 (150 ml)** | 3.5 cm | 16.5 cm | 0.22 | 13 | 11.09 |
| **AFTER 30 DAYS OF OBSERVATION** | | | | | |
| **Control -1** | 12 cm | 30 cm | 0.18 | 9 | 10.0 |
| **Control -2** | 9 cm | 32 cm | 0.15 | 8 | 9.0 |
| **T1 ( 50 ml)** | 15 cm | 28 cm | 0.20 | 15 | 16.94 |
| **T2 ( 100 ml)** | 18 cm | 35 cm | 0.19 | 16 | 15.64 |
| **T3 (150 ml)** | 14 cm | 38 cm | 0.25 | 19 | 14.55 |

# CONCLUSION

*Azospirillum* is currently one of the most broadly studiedand commercially employed PGPB.Nitrogen is available in abundance in gaseous form in the atmosphere but it is unavailable to plants unless it is reduced to ammonia. The process of reduction of atmospheric nitrogen to ammonia is carried out by prokaryotic microorganisms. *Azospirillum* has potential use as biofertilizers in agriculture mainly as a nitrogen fixer.The capability of the *Azospirillum* to extend within therhizosphere of crop recommends its capacity to get waybetter the supplement accessibility to the plants and beable to improvement the costly inorganic and organicfertilizers. Shifting to biofertilizers paves the way for a better organic environment.

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