Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 7 Issue 6 June 2023

Review Article

The Performance of Indigenous *Azospirillum* of the Plant Growth Under Pot Culture from Madurai District

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Received: April 27, 2023

Published: May 23, 2023

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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).

Keywords: *Azospirillum*; IAA; Pot Culture Experiment; Plant Root Growth

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 *Azospirillum brasilense, Azospirillum amazonense, Azospirillum irakense, Azospirillum lipoferum, Azospirillum largimobile, Azospirillum halopraeferens, Azospirillum oryzae, Azospirillum canadensis, Azospirillum doebereinerae, and Azospirillum melinis* (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 and 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 (2 ml), 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 ± 1ºC. 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 ± 1ºC 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 ether in a separating funnel and kept in refrigerator at 4ºC. 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 using spectrophotometer 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.

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 by adopting enrichment culture technique. The colony morphology of isolates on N-free malate medium was small to medium, pale white dense, spindle and transparent pale shiny white in color. All the isolates formed subsurface pellicles in NFBTB medium and turned olive green color of Bromo Thymol Blue (BTB) to brilliant blue. Formation of pellicle in NFBTB indicates the isolates seem to be *Azospirillum* spp. The pellicle formation may be considered as one of the criteria for identification. The morphological characteristics of the isolates in the present 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.

Figure 1: Isolated plate.

Figure 2: *Azospirillum* Slants.

Biochemical characterization

All the isolates were tested for biochemical characteristics. Under carbon source utilization test, the test tubes showed the appearance of turbidity which indicated utilization of glucose. Biotin requirement test shows Biotin free medium favored the growth of *Azospirillum brasilense*, *Azospirillum lipoferum* required biotin for growth. The change in color of BTB from green to yellow was recorded in the Glucose production 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.

Figure 3: Glucose peptone broth.

Figure 4: Centrifuged broth containing *Azospirillum.*

Mass production

The isolated strains were used for mass inoculums production. The isolates were stored in the refrigerator under low temperature. These strains are called starter culture. These cultures were then transferred into 100 mL, 250 mL conical flask containing N- free malic acid semisolid medium incubated into for28 ± 20ºC for one week. Then the mother inoculums were transferred into 500 mL, 1000 mL conical flask containing N- free Malic acid liquid broth (without agar). These flasks were placed in the shaker using occasional shaking for 7 days for proper aeration and agitation. After 7 days, the liquid culture containing *Azospirillum* were mixed with carrier material. Lignite powder was used as carrier. The carrier material 4 kg was mixed with 1 liter of culture broth with 2% $CaCO_3$ was mixed and then the mixture is placed in a polythene sheet and covered with polythene sheet for 24 hrs for curing. Then the mixture was pocketed into polythene bags and seal edit. The polythene bags were stored into the store room at 28 ± 20ºC. These culture pockets were used for field application.

Figure 5: Broth initially after inoculation.

Figure 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. 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.

Conclusion

Azospirillum is currently one of the most broadly studied and 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

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

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

Figure 7: *Azospirillium* inoculated Rice seedlings under Pot Culture Conditions.

Figure 8: Biometric Observation of *Azospirillum* treated Rice seedlings.

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 the rhizosphere of crop recommends its capacity to get way better the supplement accessibility to the plants and be able to improvement the costly inorganic and organic fertilizers. Shifting to biofertilizers paves the way for a better organic environment [1-10].

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