International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 317-320 www.ijprajournal.com ISSN: 2456-4494

Assessment, Isolation and Commercial Production of Azotobacter for Yield Stability

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Date of Submission: 15-07-2025 Date of Acceptance: 25-07-2025

ABSTRACT
Azotobacter is non-symbiotic, free living, aerobic nitrogen fixing bacterium found mainly in the rhizosphere and phyllo sphere soil.The soil samples were collected from The Indian Agriculture College, Radhapuram, Tirunelveli district, Tamil Nadu on 9 May 2022. The samples were evacuated from rice field and pulse field. The rhizospheric soil of 15 cm depth were collected in sterile polyethylene bags, and transported to the laboratory and stored at 4 °C until use. The study was undertaken to isolate and identifythe presence of Azotobacter in the soil of The Indian Agriculture college,Radhapuram, Tirunelveli district, Tamil Nadu.Soil samples were collected from rice and pulse field and species of Azotobacter was isolated. Waksman No 77 was the media used for isolation.The result was observed to be the appearance of round white translucent gummycolonies. Hence it was concluded that the rhizosphere soil of The Indian Agriculture College showed the presence of Azotobacter.

I. INTRODUCTION

Azotobacter is commonly found in rhizosphere and phyllosphere of plants and is very effective for the soil fertility and crop productivity. It helps in fixing nitrogen directly from the atmosphere and helps in improving the plant yield.Azotobacter is a non-symbiotic,free living, aerobic nitrogen fixing bacterium.The bacteria are gram negative and dose not absorb the stain when gram staining is performed.This characteristic differentiates it from many other nitrogen-fixing bacteria that require anaerobic or micro aerobic conditions to protect the oxygen-sensitive nitrogenase (Setubal et al., 2009). This feature also makes it an ideal candidate strain for potential co-culture with oxygenproducing phototrophs such as microalgae (Ortiz-Marquez et al., 2012). Cells show polymorphism and forms cyst accumulate poly β-hydroxybutyrate (PHB) and produces abundant gum. The main function of azotobacter in agriculture is in the fixing of atmospheric nitrogen and making it in available form for the plants. In addition, it also secrets plant growth hormones like IAA, GA, and growth factors like thiamine, riboflavin, etc…

Azotobacter was first discovered by a Dutch microbiologist and botanist Beijerinck in 1901. (Martyniuk and Martyniuk, 2003). The genus Azotobacter has 7 different species which includes Azotobacter croococcum, A. armeniacus, A. beijerinckii, A. paspali A. salinestris A. nigricansand A. vinelandii. Their size ranges from 1-2 µm wide and 2-10 µm long (Garrity et al., 2015)

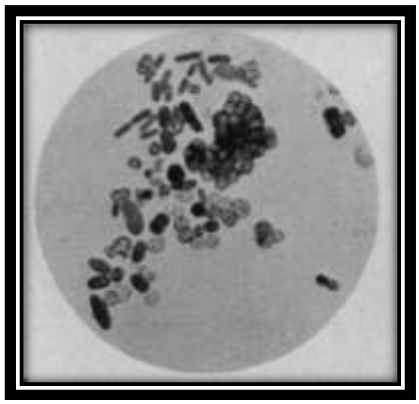

FIGURE 1 : Azotobacter population under microscope

Azotobacter is used as a biofertilizer mainly for non leguminous crop especially for rice ability to fix atmospheric nitrogen. Azotobacter is widely used as an alternative for chemical nitrogen fertilzers. The cost of cultivation is also drastically reduced without causing any degradation to the environment or soil pollution.

MATERIALS REQUIRED

1. Organic matter rich soil sample
2. Distilled water
3. Waksman No 77 media
4. Laminar air flow chamber
5. Sterile petri plate, sterile test tubes
6. Micro pipette
7. Autoclave
8. Hot air oven
9. Weighing machine

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10. Inoculation Needle

MEDIA PREPARATION

COMPOSITION:Waksman No 77 (Becking,1981)

The materials required for the preparation of 1000 ml media is as follows:

Mannitol	10.0g
Calcium Carbonate	3.0g
K_2HPO	0.5g
$MgSO_4.7H_2O$	0.2g
NaCl	0.2g
$MnSO_4.4H_2O$	Trace
N-Free washed Agar	15.0g
P^H	7.0
Distilled water	1000ml

Figure 2: Waksman No 77 media

II. MATERIALS AND METHODS

Soil Sample collection

The soil samples were collected from The Indian Agriculture College, Radhapuram, Tirunelveli district, Tamil Nadu on 9 May 2022. The samples were taken from rice field and pulse field. The rhizospheric soil of 15 cm depth was collected in sterile polyethylene bags, which were transported to the laboratory and stored at 4 °C until use.

Figure 3: soil sample

ISOLATION OF AZOTOBACTER SPP. FROM SOIL SAMPLE

SERIAL DILUTION TECHNIQUE

Serial dilution technique was adopted to isolate Azotobacter spp. 1 gm of soil was weighed from each of the sample collected and transferred to 100 ml distilled water and 10^{-2} dilution was prepared. 1 ml of this solution was added to test tube containing 9 ml of distilled water and 10^{-3} dilution was prepared of the collected soil sample.

Figure 4: Serial Dilution

PLATING TECHNIQUE (POUR PLATE METHOD)

One ml of the solution was pipetted out and transferred topetridish. The media(Waksman No 77) was melted and poured in to the Petridish at luke warm temperature and was rotated in clockwise and anti-clockwise direction for uniform distribution of the media aseptically in laminar air flow cabinet and incubated at 28°C for 3 – 4 days in the incubator. The culture plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). The observation was done on 13 /5/2022 and the result was the appearance of white translucent raised gummy colonies which was oval to round in shape.

Figure 5: pour plate method

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Figure 6: observation of pour plate method

PURE CULTURE TECHNIQUES (STREAK PLATE METHOD)

For the isolation of pure single colonies from the growth developed streak plate method was carried out. Waksman No 77 media was melted and waited till it reached Luke warm temperature in the laminar air flow chamber and checked by touching the conical flask in the dorsal side of the hand. Once it attained Luke warm temperature the media of 10-15 ml was transferred to petri plates. After the media solidified with the help of sterilized inoculation needle the colonies where streaked into the plates in all four directions. The petri plates where incubated at 28°C for 3-4 days. The observation was done on 20/5/2022 and the result was the appearance of white translucent raised gummy colonies

FIGURE 7: Observation of streak plate method

PRESERAVTION TECHNIQUES OF AZATOBACTER

SLANT CULTURE TECHNIQUE

Agar slant is prepared mainly for the maintenance of pure culture for a moderately long time with the help of refrigeration for 3 months. The main objective of using a slant is to minimise the attack of contamination.

Waksman no 77 media is melted, 5ml media is measured and transferred to test tubes. Cotton is plugged into the test tube mouth and covered with brown paper.The test tubes are autoclaved at 121°c 15 lb pressure for 20 minutes.The test tubes are placed in a slant position and slants are prepared.After the slants solidify sterile inoculation needles are used for inoculation from pure culture obtained in streak plate method in laminar air flow chamber. After 3 -4 days of incubation white translucent colonies was observed.Finally, the slant culture was covered with klin film and was kept in refrigerator for preservation on 19/5/2022.

Figure No 8: Observation in slant culture

CONFIRMATION STUDIES ON AZOTOBACTER

Based on the morphological characters observed, small, round, and white slimy colony was identified as Azotobacter bryophyllii.

Table 1 : Isolation of Azotobacter from native soil of TIAC .

SI. No	Isolate Number	Location (Tirunelveli Dt.)	Crop
I	Azotobacter		
1	RTIAC-1 *	Radhapuram	Rice

***Isolates used in the present investigation**

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Table 2: Characterisation of Azotobacter

SI.NO	Characters	Result
1	Microscopic examination	Rod
2	Morphology	White Slimy colonies
3	Gram Reaction	Negative
4	Pigment production	Absent

III. RESULT

Azotobacter isolate was isolated from rice rhizosphere soil collected from Radhapuram. The isolate Azotobacter was characterized and their result are summarized in Table 2. The isolate was rod shaped gram negative, white slimy colonies and without any pigments. The isolation was identified as Azotobacter bryophyllii.

IV. CONCLUSION

From the study that was undertaken at The Indian Agriculture College it is concluded that Azotobacter is present in the native soil in the area under study. The species identified where not pigmented even after 3 - 4 days of incubation. Azotobacter can be commercially produced in large scale and used as a biofertilizer and the cost of cultivation could be reduced. Azotobacter species are gram negative and can fix atmospheric nitrogen. The importance of Azotobacter is to improve the soil fertility and to improve the economical yield for the farmer and stabilize the income eventually.

REFERENCE

[1]. Garrity, G. M., Bell, J. A., & Lilburn, T. (2015). Alphaproteobacteria class. nov. Bergey's Manual of Systematics of Archaea and Bacteria, 1-1.

[2]. Martyniuk, S., & Martyniuk, M. (2003). Occurrence of Azotobacter spp. in some Polish soils. Polish Journal of Environmental Studies, 12(3), 371-374.

[3]. Ortiz-Marquez, J. C. F., Do Nascimento, M., Dublan, M. D. L. A., & Curatti, L. (2012). Association with an ammonium-excreting bacterium allows diazotrophic culture of oil-rich eukaryotic microalgae. Applied and environmental microbiology, 78(7), 2345-2352.

[4]. Setubal, J. C., Dos Santos, P., Goldman, B. S., Ertesvag, H., Espin, G., Rubio, L. M., ... & Wood, D. (2009). Genome sequence of Azotobacter vinelandii, an obligate aerobe specialized to support diverse anaerobic metabolic processes. Journal of bacteriology, 191(14), 4534-4545.