



PRODUCTION OF INDOLE ACETIC ACID BY *AZOTOBACTER* STRAINS ASSOCIATED WITH MUNGBEAN

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Abstract

A total of 16 isolates belonging to *Azotobacter* spp. were isolated from different rhizospheric soils collected from various districts of Uttar Pradesh. These test isolates were biochemically characterized and screened for their plant growth promoting trait *i.e.* indole acetic acid production (IAA). The production of IAA was shown in all isolates of *Azotobacter* in the range of 42.80-82.00 µg/ml. Among *Azotobacter* isolates, Azt-4 and Azt-6 produced highest amount (82.00 µg/ml) of IAA in the broth culture medium.

Key words : PGPR, mungbean, indole acetic acid.

Introduction

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere at root surfaces and in association with root which can improve plant growth directly and or indirectly, in last few decades a large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacteria*, *Burkholderia*, *Bacillus* and *Serratia* have reported to enhance plant growth, Plant Growth Promoting Rhizobacteria (PGPR) are considered to promote plant growth directly or indirectly. PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (PGPR) (auxins, gibberellins, ethylene etc.), siderophore, HCN and antibiotics (Arshad and Frankenberger, 1992). Indole acetic acid (IAA) is one of the most physiologically active auxins. *Azotobacter* secreted IAA into culture media and significantly increased the dry weight of leaves and roots of several plant species following root treatment (Barea and Brown, 1974). In the view of above facts, the indole acetic acid producing *Azotobacter* isolates have been isolated from mungbean rhizosphere screened for their ability to produce Indole Acetic Acid.

Materials and Methods

Isolation *Azotobacter* strains from mungbean rhizosphere

The soil samples were taken from various locations of Uttar Pradesh for isolation of *Azotobacter* strains. The soil samples were isolated by plating serial dilution of these soil samples on the Jensens agar medium. Bacterial colonies with milky growth that turned brown later were isolated and purified on the respective medium and identified as *Azotobacter* spp. by cultural, morphological and biochemical tests as described in Bergeys manual of determinative bacteriology (Holt *et al.*, 1994).

Bio-assay of *Azotobacter* strains for indole acetic acid

All the test strains were screened for IAA production by the modified method as described by Loper and Scrowth (1986). The *Azotobacter* cultures were grown for 72 hours on the nutrient media at 28°C on rotary shaker. The fully grown cultures were centrifuged at 10000 g for 15 min. The 2 ml of supernatant was mixed with 2-3 drops of O-phosphoric acid and 4 ml of salkouski reagent solution (1 ml of FeCl₃ 0.5M mixed in 50ml of 35% HClO₄). The samples were incubated for 25 minutes at room temperature. The development of pink color was observed and optical density was taken at 530nm with help of spectrophotometer (table 1). The concentration

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Table 1 : Production of Indole acetic acid (IAA) by *Azotobacter* isolates grown in respective medium.

S. no.	Isolates	IAA production ($\mu\text{g/ml}$)
1.	Azt-1	65.55
2.	Azt-2	42.12
3.	Azt-3	81.43
4.	Azt-4	65.33
5.	Azt-5	61.50
6.	Azt-6	82.20
7.	Azt-7	76.20
8.	Azt-8	51.03
9.	Azt-9	62.40
10.	Azt-10	54.70
11.	Azt-11	65.06
12.	Azt-12	62.16
13.	Azt-13	69.96
	SE (treatment mean)	1.840383
	CD at 5%	5.351139
	CV	4.935205

of IAA produced by cultures was measured with the help of standard graph of IAA obtained in the range of 20-200 microgram per ml.

Results and Discussion

Isolation and biochemical characterization

On the basis of cultural, morphological and biochemical characteristics, a total of 16 bacterial strains were isolated and identified as *Azotobacter* spp. as described in Bergeys manual of determinative bacteriology (Holt *et al.*, 1994). The *Azotobacter* spp. strains from rhizosphere of different crops were isolated and extensively studied by Ahmad *et al.* (2005), Ahmad *et al.* (2008, 1997) and Joseph *et al.* (2007).

Bio-assay of *Azotobacter* strains for indole acetic acid

A total of 16 *Azotobacter* isolates were selected and tested for quantitative IAA production. The production of IAA was recorded in all isolates of

Azotobacter in the range of 42.80-82.00 $\mu\text{g/ml}$. Among *Azotobacter* isolates, Azt-4 and Azt-6 produced highest amount (82.00 $\mu\text{g/ml}$) of IAA in the broth culture medium (table 1). Ahmad *et al.* (2005) reported that *Azotobacter* spp. produced 38.82 $\mu\text{g/ml}$ IAA in culture medium supplemented with Tryptophan at the rate of 5mg/ml. Aris *et al.* (2011) examined the *Bacillus* species from the rhizosphere of soybean plant of Cirebon, Indonesia, and found 76.3% *Bacillus* strains produced indole acetic acid (IAA). The findings of present investigation are outstanding in reference to earlier reports.

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