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## Screening of *Azotobacter* isolate for PGT properties

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**Abstract-** Maize rhizosphere soil was collected from 10 villages of Madhepura district. Bacteria from rhizosphere soil were isolated. Altogether, seven isolates were obtained. All belonging to genus *Azotobacter*. Isolates were characterized on the basis of their morphological and biochemical characters as described in Bergey's manual of determinative bacteriology. All isolates were able to solubilize phosphate as tested in PVK and NBRIP medium. The solubilization index ranged from 13.7 to 24.9. The maximum was in MAZ<sub>5</sub> and minimum in MAZ<sub>6</sub>. Siderophore production and HCN production was positive in all isolates. The production of Indole acetic acid varied from 30.55 to 48.62 when 5mM L-tryptophan was supplemented in the medium.

**Key words:** PVK, NBRIP, Solubilization Index, Siderophore.

### INTRODUCTION

Maize is one of the most versatile emerging crops having wider adaptability under varies agro-climatic conditions. In India Maize is the third most important crop after Rice and Wheat. It is cultivated in all status of the country for various purposes including grain, fodder, green cobs, sweet corn, baby corn, popcorn, etc. The predominant maize growing states are AP, MP, UP, Bihar, Karnataka, Maharashtra and Rajasthan. For the production of higher yield appropriate fertilization is required.

Growth promoting Rhizobacteria have been used to enhance the crop yield. These bacteria directly involved in the uptake of Nitrogen through biological Nitrogen fixation, Synthesis of Phytohormones, Solubilization of Phosphorus and Potash and production of Siderophores. The beneficial

effect of *Azotobacter* on yield of maize was reported by numerous authors (Biari *et al.* 2008, Gholami *et al.* 2009, Jarak *et al.* 2012)<sup>1-3</sup> yield increased by *Azotobacter* inoculation is due to nitrogen fixation, production of phytohormones and antipathogenic activity.

### MATERIALS & METHODS

Rhizosphere soil samples were collected from Maize plants grown in different fields of ten villages of Madhepura district (Murliganj, Chousa, Manpur, Bhelva, Budhma, Lalpur, Chikni, Sukhasan, Murho, Bakhri). Samples collected from these villages. Seven isolates MAZ<sub>1</sub>, MAZ<sub>2</sub>, MAZ<sub>3</sub>, MAZ<sub>4</sub>, MAZ<sub>5</sub>, MAZ<sub>6</sub>, MAZ<sub>7</sub> were obtained. pH, Nitrogen %, humus, P, K, content of soil were measured as per standard methods. The isolated bacteria was identified by morphological character and biochemical tests. For morphological characterization, Gram staining was made. The shape, color, margin, nature and texture

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of colony was examined. In biochemical test, Citrate utilization test, Methyl red test, Vages-Proskaur test, Indole test, Catalase test, Glucose test, Lactose test, Sucrose test, Oxidase test and Urease test were performed.

**Phosphate Solubilization Test:**

For Phosphate solubilization test serial dilution of culture from Ashbey’s medium was prepared in autoclaved distilled water and plated on PVK Agar medium supplemented with ICP Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> and NBRIP medium. The culture was insulated at 30°C for 7 days. Solubilization was calculated by measuring diameter of colony and holozone using the formula:

$$SI = \frac{\text{Colony diameter} + \text{Clearing zone}}{\text{Colony diameter}} \times 100$$

**Siderophore Production:** Siderophore production was assayed on Chrom-Azurol S medium as prescribed by Milagres *et al.*(1999)<sup>4</sup>.

**HCN Production:** HCN production was tested on HCN induction medium.

**Supplemented with Glycine:**

**IAA production:** For estimation of IAA production 2 days old culture was inoculated in N<sub>2</sub>-free liquid culture medium supplemented with L-Tryptophan and incubated for 48 hrs. at 30°C. Salkowski reagent was mixed and the intensity of developed color was measured at 530nm in spectrophotometer.

**RESULT**

Altogether, seven isolates were obtained from Rhizosphere soil samples of Maize plants obtained from different villages of Madhepura district. pH of the soil, Nitrogen content, available nitrogen and available Phosphorus from each soil sample was determined. The maximum pH (7.5) was recorded from the soil sample of Lalpur village and minimum pH (6.5) was recorded from the soil sample of Murliganj village. Nitrogen content and available Nitrogen was maximum in Murho village (0.089% and 6.4ppm) while the available Phosphorus was maximum in the soil sample of Bakhri (9.8 ppm). The result is recorded in table no. 01.

Among all seven isolates obtained from Maize rhizosphere were identified on the basis of their morphological and biochemical characters as described in Bergey’s manual of determinative bacteriology. All isolates were Gram negative cocci forming regular or irregular colony, their morphological and biochemical characters

are represented in Table no. 02 & 03. The carbon utilization of all isolates is described in Table no. 04.

**Table 1- pH, N<sub>2</sub>-content, K-content of soil samples**

Sl. No.	Name of Villages	pH	N <sub>2</sub> Content (%)	Available N <sub>2</sub> (ppm)	Available P (ppm)
1	Murliganj	6.5	0.087	4.2	8.7
2	Chousa	6.9	0.067	5.2	3.2
3	Manpur	7.2	0.076	5.1	8.2
4	Bhelva	6.8	0.076	2.9	4.8
5	Budhma	7.1	0.053	4.6	5.3
6	Lalpur	7.5	0.066	4.2	6.5
7	Chikni	6.9	0.073	4.5	7.7
8	Sukhasan	7	0.063	4.6	5.5
9	Murho	7.3	0.089	6.4	6.5
10	Bakhri	6.7	0.083	2.83	9.8

**Table No. 02: Morphological and Characters of isolates**

Sl. No.	No. of Isolates	Shape of Colony	Colony size	Cell shape	Gram staining
1	MAZ <sub>1</sub>	Regular	Small	Oval	Negative
2	MAZ <sub>2</sub>	Regular	Medium	Oval	Negative
3	MAZ <sub>3</sub>	Irregular	Small	Oval	Negative
4	MAZ <sub>4</sub>	Regular	Small	Oval	Negative
5	MAZ <sub>5</sub>	Irregular	Small	Oval	Negative
6	MAZ <sub>6</sub>	Irregular	Medium	Oval	Negative
7	MAZ <sub>7</sub>	Regular	Medium	Oval	Negative

**Table No. 03: Biochemical tests**

No. of Isolates	Oxidase	Catalase	Indole	Urease	Voges Proskauer
MAZ <sub>1</sub>	+	+	+	+	-
MAZ <sub>2</sub>	+	+	+	+	-
MAZ <sub>3</sub>	+	+	+	-	-
MAZ <sub>4</sub>	+	+	+	-	-
MAZ <sub>5</sub>	+	+	+	+	-
MAZ <sub>6</sub>	+	+	+	+	-
MAZ <sub>7</sub>	+	+	+	+	-

**Table No. 04: Carbon utilization**

No. of Isolates	Glucose	Maltose	Sucrose	Mannitol	Arabinose
MAZ <sub>1</sub>	+	-	+	+	-
MAZ <sub>2</sub>	-	+	+	+	-
MAZ <sub>3</sub>	-	+	-	+	-
MAZ <sub>4</sub>	+	+	-	+	-
MAZ <sub>5</sub>	+	-	+	+	-
MAZ <sub>6</sub>	-	-	+	+	-
MAZ <sub>7</sub>	+	+	-	+	-

Table No. 04: Carbon utilization

No. of Isolates	P. Sol.		SI	Siderophore	HCN	IAA production( $\mu\text{g/ml}$ )		
	PKV	NBRIP				mM L-tryptophan		
						0	2.5	5
MAZ <sub>1</sub>	+	+	15.5	+	-	3.56	21.05	31.56
MAZ <sub>2</sub>	+	+	17.3	+	+	2.98	20.35	31.24
MAZ <sub>3</sub>	+	+	21.5	+	+	4.5	30.26	41.06
MAZ <sub>4</sub>	+	+	20.6	+	-	5.06	36.42	45.85
MAZ <sub>5</sub>	+	+	24.9	+	+	4.13	27.56	37.95
MAZ <sub>6</sub>	+	+	13.7	+	+	3.22	20.75	30.55
MAZ <sub>7</sub>	+	+	22.6	+	+	5.45	38.65	48.62

For the screening of Plant Growth Promoting Rhizobacteria (PGPR) their solubilizing ability was tested in PVK medium and NBRIP medium. The clearing zone and colony diameter were measured and solubilization index (SI) was calculated. The SI value was tabulates in Table no. 05. All isolates shows positive result in both PVK and NBRIP medium. Siderophore production was assayed on Chrome-azurool S medium. All isolates showed positive result. HCN production was also tested in HCN induction medium supplemented with Glycine. Two isolates were negative while all other five isolates were positive. Test for indole acetic acid production without L-tryptophan and with tryptophan were performed. The tryptophan was supplemented in a concentration of 2.5mM and 5mM concentration in Nitrogen free medium. The production of IAA without L-tryptophan was very poor while the production was maximum in 5mM concentration. The result is tabulated in Table no. 05.

#### CONCLUSION

A large no. of bacteria are capable of solubilizing phosphate in soil. In the present study, seven isolates were identified from the rhizosphere of Maize plant collected from different villages of Madhepura district. All isolates were tested for their ability to solubilize phosphate. For this purpose, they were tested in PVK and NBRIP medium. All isolates showed positive result. The solubilizing index was maximum for isolate number MAZ<sub>5</sub>(24.9) and minimum for MAZ<sub>6</sub>(13.7). All isolates were cultured in Ashby's medium and their morphology was studied microscopically. Cells were coccoid in all isolates. The shape of colony was regular in MAZ<sub>1</sub>, MAZ<sub>2</sub>, MAZ<sub>4</sub> and MAZ<sub>7</sub> and irregular in MAZ<sub>3</sub>, MAZ<sub>5</sub> and MAZ<sub>6</sub>. Colony size was small or medium. In all isolates, cells were coccoid and Gram negative. The biochemical test oxidase, catalase, Indole, Urease and VogesProskauer were performed. The

Voges Proskauer test was negative in all isolates while Oxidase, Catalase and Indole test were positive. The Urease test was negative in Isolate no. MAZ<sub>3</sub> and MAZ<sub>4</sub>.

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