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Isolation and Screening of Drought Tolerent *Azotobacter* and PSB from Sorghumrhizosphere *(Sorghum bicolor* L. Moench*)*

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***ABSTRACT*:** *The present investigations entitled "Isolation and screening of drought tolerant* Azotobacter *and PSB from sorghum rhizosphere* (Sorghum bicolor *L. Moench)" were conducted at Plant pathology, College of Agriculture, Pune, during 2011-2013. The studies were carried out to know the drought tolerance of* Azotobacter *and PSB isolates from dryland sorghum rhizospheric soil .Therefore, rhizosphere soil samples of sorghum from ten different locations in Solapur district were collected for isolation of* Azotobacter *and PSB. Out of ten soil samples, two did not show the presence of 'N' fixers and PSB. Eight isolates were studied for their morphological and cultural characteristics. All 'N' fixing isolates were Gram negative and PSB isolate were Gram positive. The identity of 'N' fixing isolates was confirmed as* Azotobacter. *Old* Azotobacter *cultures were observed to produce light brown to dark blackish brown pigmentation. The screening experiment reveled that,* Azotobacter *isolate 5 had the higest 'N' fixing ability (31.7 mg / g of sucrose) and PSB isolate 2 had maximum 'P' solubilising ability (40.4%) and zone of clearance (14 mm).*

Keywords : *Drought tolerance,* Azotobacter*, PSB, Sorghum.*

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is one of the world's most important nutritional dry land cereal crop and also the major staple food crop of millions of people in semi-arid tropics (SAT.) It is one of the most important cereal crop in the world agricultural economy both as food for man and feed for animals.

Sorghum variety *i.e.,* Maldandi-M-35-1 is evolved at the Agricultural Research station, Mohol (District-Solapur) and is very popular for drought tolerance. It is recommended for major parts of Maharashtra and Karnataka particularly for sowing in rabi season. It is normally grown under stored and receding soil moisture conditions with increasing temperature after flowering.Thus, it experiences both soil and atmospheric water deficit (drought). The limited availability of water causes moisture stress which affects various metabolic processes of the plant.

Drought adversely affects some of the important physiological, biophysical and biochemical processes of the plants, like chlorophyll destruction, enzymatic activities and protein synthesis. Some of the micro-organisums are known to be better adapted to drought condition have the ability to function normally during and after stress period. The major challenges in drought prone areas is to establish ways and means by which drought suseptability in crop can be minimized.

1. To isolate the strains of *Azotobacter* and PSB from the rhizosphere of dry land sorghum.
2. To study the efficiency of *Azotobacter* and PSB in vitro condition.

MATERIALS AND METHODS

For isolation of drought tolerant*Azotobacter* and phosphate solubilising bacteria soil samples were collected from fields of dry land Sorghum rhizosphere from different tehsils of Solapur district during Rabi season 2011.

Isolation of *Azotobacter*

Isolation of *Azotobacter* from the soil samples collected from different places was done following standard enrichment culture and streaking technique.

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Thousand ml nitrogen free Jensen's broth was prepared and dispensed 100 ml each in 250 ml conical flasks. After cooling 1 gm soil samples was inoculated in each conical flask which were placed on incubator shaker for 7 days at 28°C± 2°C temp. providing aerobic condition to enhance the growth of bacterial cells.

A loopful of inoculums from the pellicles of each flask was streaked on Jensen's agar plates to obtain well isolated colonies and incubated in BOD at 28°C ± 2°C temp. in inverted position for 5 days.

The plates were observed for development of typical *Azotobacter*colonies.

Isolation of PSB

Isolation of PSB from soil samples collected from different places was done by enrichment culture and streaking technique described by Pikovaskia (1948).

After incubation, at desired temp. (28°C ± 2°C) the plates were observed for the bacterial colonies with zone of clearance and transferred on the slants.

Purification and Further Multiplication of Isolates

Loopful of growth from the slant was streaked again on fresh plates of nitrogen free Jensen's agar medium/ Pikovaskia's agar medium incubated for independent colony development at 28°C ± 2 in incubator. Thereafter every isolate was transferred on fresh slants, maintained in pure state and preserved for further studies.

Identification of Cultures

Cultural tests

Colony characters

Plates of nitrogen free Jensen medium and Pikovaskia's medium were streaked and incubated for a week and well-seperated colonies were examined daily for various colony characters. The colony attributes viz.,maximum diameter, opacity, edge, elevation, consistency, and surface appearance and Zone of clearance were recorded with the descriptive terms defined by SAB(1957).

Pigmentation

Plates of nitrogen free Jensen medium were streaked and incubated for two weeks and well-seperated colonies were examined daily for pigmentation and colony colours have been distinguished by using colour chart.

Growth characteristics in broth

All the isolates were grown for a week in 250 ml of Jensen's broth in conical flask separately. The cultural attributes of the growth were recorded in accordance with terms defined in SAB (1957).

Morphological Tests

Gram staining

A thin bacterial smear from one-day-old culture was made on a clean slide, fixed by gentle heating and Gram stained as described by Kopeloff and Berman (SAB, 1957) and observed through microscope under oil immersion.

Screening of *Azotobacter* and PSB isolates for nitrogen fixation and 'P' solubilization:

Eight isolates of *Azotobacter* obtained from ten soil samples collected from various locations were multiplied on nitrogen free medium. The amount of nitrogen fixed by the *Azotobacter* isolates during incubation in broth culture was estimated by Micro-Kjeldhal's method (Jackson, 1976).The efficient isolates were tested under pot culture trial for assessing their benefit.

Eight PSB isolates were multiplied in Pikovaskia's broth and amount of 'P' solubilized was estimated at the end of 4 days inoculation by Olsen's method.

RESULTS AND DISCUSSION

Occurrence of *Azotobacter* and PSB

Ten soil samples were collected from rhizosphere of dryland sorghum from various places. *Azotobacter*and PSB were isolated from these samples on Jensen's agar medium and Pikovaskia's medium on the basis of colony characters.

Pikovskaya (1948), Quagliano *et al*. (1994), Mattos *et al*. (1997), El-Dsouky *et al*. (2003) andChaiharn *et al*. (2008) reported the *Azotobacter* and PSB isolated from rhizospheric soil and screened for growth promoting properties.

***Azotobacter* and PSB isolates**

Eight cultures of each isolates were obtained from ten soil samples and purified and maintained in pure form. *Azotobacter*were labelled as *Azotobacter* isolate 1, *Azotobacter* isolate 2, ..., *Azotobacter* isolate 8and PSB isolates as PSB isolate 1, PSB isolate 2, ... PSB isolate 8.

Table 1
Cultural characteristics of*Azotobacter* isolates

Azotobacter isolates	*Colony characters*			*Growth rate*	*Colony diameter*	*Pigment production*
	Form	*Elevation*	*Margin*			
Azotobacter1	Circular	Raised	Entire	Fast	7	Blackish
Azotobacter2	Circular	Raised	Lobate	Moderate	4	Brown
Azotobacter3	Irregular	Raised	Lobate	Slow	2	Light brown
Azotobacter4	Circular	Raised	Entire	Moderate	5	Brown
Azotobacter5	Irregular	Raised	Lobate	Fast	9	Blackish
Azotobacter6	Circular	Raised	Entire	Moderate	6	Light brown
Azotobacter7	Circular	Raised	Entire	Slow	3	Light brown
Azotobacter8	Irregular	Raised	Lobate	Slow	3	Light brown

Identification of Isolates

The *Azotobacter* isolates 1, 2, 4, 6, 7 were observed to be circular and *Azotobacter* isolates 3, 5, 8 as irregular. The colony elevation of all isolates were Raised. Types of margin revealed that *Azotobacter*isolates 1, 4, 6, 7wereentire and *Azotobacter* isolates 2, 3, 5, 7as lobate. The growth study of the isolates on Jensen's medium 7 days after inoculation showed that *Azotobacter* isolates 5 and 1were fast growers as exhibited by the colony diameter of 7 to 9 mm. The moderate growers *viz*., *Azotobacter* isolates 2, 4, and 6had an average colony diameter of 4 to 6 mm. The slow growers exhibited the growth in terms of colony diameter of 2 to 3 mm. *viz. Azotobacter* 3, 7 and 8.

Two strains *viz*., *Azotobacter* isolates 2 and 4 recorded brown pigment while *Azotobacter* isolates 3, 6, 7, and 8 produced light brown pigment, further dark blackish brown pigment was also observed to be produced by the strains *viz*., *Azotobacter* isolates 1, 5, (Table 1). The black brown water insoluble pigment was produced by aged cultures of *Azotobacter* which attributed to the secretion of melanin. This melanin is formed as a result of oxidation of tyrosine, a copper containing amino acids.

The PSB isolates were under study exhibited the following of colony characters. The isolates PSB isolates 1, 4, 5, and 7were observed to be irregular and PSB 2, 3 and 8 as rhizoid. The colony margin of all isolates werelobate and type of elevation were raised.

The growth study of the isolates on Pikovaskia's medium 7 days after inoculation showed that P_2 was fast growers as exhibited by the colony diameter of 9 mm. The moderate growers *viz*., PSB 1, 4, and 5 had an average colony diameter of 5 to 7 mm. The slow growers exhibited the growth in terms of colony diameter of 2 to 3 mm. *viz*., PSB 3, 6, 7, and 8 respectively (Table 2)

Growth Characteristics in Broth

The Pellicle growth were exhibited by the all isolates of *Azotobacter* and PSB in broth culture.

Morphological Test

Gram Staining

The gram staining reactions of *Azotobacter* and PSB were observed to be gram -ve and gram +ve respectively.

Screening of *Azotobacter* and PSB Isolates for Nitrogen Fixation and 'P' Solubilization in Invitro

The amount of nitrogen fixed by the *Azotobacter* isolates during incubation in broth culture.

Table 2
Cultural characteristics ofPSB isolates

PSB isolates	*Colony characters*			*Growth rate*	*Colony diameter*
	Form	*Elevation*	*Margin*		
PSB isolate 1	Irregular	Raised	Lobate	Moderate	7
PSB isolate 2	Rhizoid	Raised	Lobate	Fast	9
PSB isolate 3	Rhizoid	Raised	Lobate	Slow	2
PSB isolate 4	Irregular	Raised	Lobate	Moderate	5
PSB isolate 5	Irregular	Raised	Lobate	moderate	6
PSB isolate 6	Rhizoid	Raised	Lobate	Slow	6
PSB isolate 7	Irregular	Raised	Lobate	Slow	3
PSB isolate 8	Rhizoid	Raised	Lobate	Slow	3

Table 3
Nitrogen fixed in culture broth (mg/g of sucrose consumed The amount of nitrogen fixed by the Azotobacter isolates during incubation in broth culture.

Azotobacter isolates	*'N' fixed in 'N' free broth*
Azotobacterisolate 1	29.2
Azotobacterisolate 2	20.1
Azotobacterisolate 3	21.0
Azotobacterisolate 4	26.6
Azotobacterisolate 5	31.7
Azotobacterisolate 6	24.3
Azotobacterisolate 7	19.1
Azotobacterisolate 8	16.7

'N' fixation by different *Azotobacter* isolates exhibited a great variation in the range of 16.7 to 31.7 mg/g of sucrose consumed; the highest amount 'N' was fixed by the isolate number *Azotobacter* isolate 5.

Uppal and patel (1947), Rao and Venkateswarlu (1982), Saxena (1984) reported that, recorded the efficiency of *Azotobacter* isolates for 'N' fixation in 'N' free broth medium.

The variation in 'P' solubilising efficiency of different isolates were noted in the range of 21.45 to 40.43%. The highest % of 'P' solubilisation was recorded by isolate number 'PSB isolate 2'.

These results are in confirmed with the findings of Pikovskaya (1948), Bilolikar (1996), Nautiyal (1999), Sanjay Kumar *et al*. (2005) reported that, efficiency of phosphate solubilizing bacteria ranged from 10 to 22 mg P solubilized / 100 ml of Pikovaskaya's broth and bacterial colonies forming halo zones were considered to be phosphate solubilizers.

Table 5
% 'P' solubilisation in culture broth and diameter of zone of clearance of PSB isolates:
The % 'P' solubilisation by PSB isolates during incubation in broth culture.

PSB isolates	*% 'P' solubilisation*	*Solubilisation zone (mm)*
PSB isolate 1	31.7	11
PSB isolate 2	40.4	14
PSB isolate 3	39.0	10
PSB isolate 4	30.4	9
PSB isolate 5	32.9	10
PSB isolate 6	20.0	10
PSB isolate 7	19.5	9
PSB isolate 8	21.4	10

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