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SCREENING OF *AZOTOBACTER* ISOLATES OF WHEAT RHIZOSPHERE FOR PHOSPHATE SOLUBILIZING ACTIVITY AND SALT STRESS

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ABSTRACT

Numerous species of soil bacteria that flourish in the rhizosphere of plants may stimulate Plant growth by various mechanisms. These rhizobacteria are collectively known as plant growth promoting rhizobacteria (PGPR). The plant growth promoting traits like production of indole-3-acetic acid (IAA), gibberellins, siderophore, and phosphorus solubilization etc. in the rhizobacteria are the most common. The Phosphorus Solubilizing bacteria are used as plant growth promoting bacteria (PGPB) in agriculture. An experiment was carried out in department of plant molecular biology and genetic engineering, Narendra Deva University of Agriculture and Technology Kumarganj Faizabad during 2012-13 to search of phosphorus solubilizing Azotobacter strains of wheat plants grown in various locations of Uttar Pradesh. A total of sixteen strains were isolated on the Jensen's medium and identified as Azotobacter spp. Phosphorus solubilizing capabilities were demonstrated by the formation of clearing zone on the Pikovaskya medium. Out of 16 Azotobacter strains, only 10 strains were found able to solubilize phosphate in the medium. The isolate Azt-sut2 and Azt-lko2 showed high level of phosphate solubilization while Azt-uno1 and 2, Azt-mth2 and Azt-gnd2 was medium phosphate sulubilizer and Azt-fzb2, Azt-sut1, Azt-gnd1 and Azt-knp1recorded with low phosphate solubilization activity. All the Azotobacter isolates showed tolerance up to 8% NaCl concentration whereas Azt-sut2, Azt-gnd1, Azt-gnd2, Azt-uno2 and Azt-mth2 could tolerate even 10% concentration of NaCl.

Keywords: *Azotobacter*, wheat rhizosphere, screening, phosphate solubilization

INTRODUCTION

The wheat crop is mainly cultivated under rain-fed condition where precipitation is less than 900 mm annually. Salinity is one of the major constraints which hamper wheat production in India. The use of plant growth promoting rhizobacteria (PGPR) may prove useful for developing strategies to facilitate wheat growth in saline area .The rhizobacteria exert plant growth promoting effect as by virtue of plant growth promoting attributes *viz*. production of indole-3-acetic acid (IAA), gibberellins, siderophore, and phosphate solubilization *etc.* Application of the rhizobacteria in crops like wheat and rice in saline area is inexpensive, simple to use and have no adverse effect on land (Upadhyay *et al.* 2012, Nakhanpote *et al.* 2015). Phosphorus is by far the least mobile and is the most limiting factor for plant growth after nitrogen compared with the other major nutrients. PSB often produce phosphatases and organic acids to facilitate P dissolution from P compounds. A vast proportion of the applied P has been bounded in phosphates in the soil with iron, aluminum and calcium *etc*. The fixed form of P is not efficiently taken up by the plants and thus causes many environmental problems such as soil salinity and eutrophication (*Zhao et al.* 2011). It has already been reported that plant growth promoting rhizobacteria (PGPR) including phosphate-solubilizing microorganisms (PSMs) are able to solubilize the unavailable forms of P in soil by acidification, chelation, and exchange reaction in the soil environment. The microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to plants. The rhizospheric phosphate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture. The most efficient PSMs belong to genera *Bacillus*, *Azotobacter* and *Pseudomonas* amongst bacteria, and *Aspergillus* and *Penicillium* amongst fungi. The application of PSMs and PGPRs can reduce P application by 50% without any significant reduction of grain yield in corn (Saharan and Nehra**,** 2011). and wheat (Chaudhary *et al.* 2013). Nitrogen fixing and phosphate-solubilizing bacteria are the two main groups of plant growth-promoting bacteria.These bacteria would be used on crops to reduce the rate of fertilizers. In our study, an effort has been made to characterize the P-solubilizing capabilities of *Azotobacter* isolates of wheat rhizosphere grown under saline conditions.

MATERIALS AND METHODS

Isolation of *Azotobacter* isolates: The rhizospheric soil samples of wheat during flowering stage were collected in sterile plastic bags from eight different salt affected district *viz*. Faizabad (fzb), Sultanpur (sut), Gonda (gnd), Barabanki (bbk), Lucknow (lko),

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Unnao (uno), Kanpur Dehat (knp) and Mathura (mth) of Uttar Pradesh. Rhizospheric soil was separated from roots of wheat with the help of brush in a petriplate. One gram soil of each sample was placed in 10 ml sterile water. Serial dilution were made up to 10^{-4} from all eight soil samples and 10^{-3} and 10^{-4} dilution were taken for spread plating on Jensen's medium with pH 7.0. The plates were incubated at 28 ^{0}C for 96 hours.

Biochemical characterization and identification of *Azotobacter* strains: The strains were identified by physiological and biochemical methods as per method described in Bergey's manual of Systematic Bacteriology (Claus and Berkeley, 1986).

Phosphate solubilization: All the *Azotobacter* isolates were spot inoculated on Pikovskaya's agar medium (Pikovskaya, 1948) and incubated at 30 ^{0}C up to 5 days. Phosphate solubilization activity was determined by development of clearing zone around the inoculated spot. Isolates exhibiting clearing zone of varying diameter on Pikovaskya's agar plate were considered as positive for phosphate solubilization (Bromsiri and Bromsiri 2010, Wahyudi *et al.*, 2011) .

Salt tolerance: Pure culture of all *Azotobacter* isolates were streaked on nutrient agar medium amended with 3% to 10% NaCl concentration. Control plates without NaCl amendment were also included for all isolates. All plates were incubated at 28 ^{0}C for 48 hours and observed for presence and absence of growth.

RESULTS AND DISCUSSION

Isolation and Biochemical characterization of *Azotobacter* isolates

Eight soil samples were collected from rhizosphere of wheat grown in different district of Uttar Pradesh. In this study, a total 16 *Azotobacter* isolates were obtained from the rhizosphere of wheat. The isolates were characterized on the basis of physiological, biochemical and morphological features, Isolates of *Azotobacter* species from rhizosphere of different crops were widely studied by Joshi *et al.*, (2011) and Chaudhary *et al.* (2013).

Table 1: Screening of *Azotobacter* isolates for Phosphate solubilization

Name of isolate	High Solubilisation	Medium Solubilisation	Poor Solubilisation	No Solubilisation
Azt-fzb-1	-	-	-	-
Azt-fzb-2	-	-	+	-
Azt--sut-1	-	-	+	-
Azt--sut-2	+++	-	-	-
Azt -gnd-1			+	
Azt -gnd-2	-	++	-	-
Azt -bbk-1	-	-	-	-
Azt -bbk-2	-	-	-	-
Azt -lko-1	-	-	-	-
Azt -lko-2	+++	-	-	-
Azt -uno-1	-	++	-	-
Azt -uno-2	-	++	-	-
Azt -knp-1	-	-	+	-
Azt -knp-2	-	-	-	-
Azt -mth-1	-	-	-	-
Azt -mth-2	-	++	-	-

Phosphate solubilization and Salt tolerance

Each of isolates was screened for phosphate solubilization and salt tolerance. Ten isolates were found able to solubilize phosphate on Pikovskaya's medium plates at 30 ^{0}C (Table 1). The intensity of phosphate solubilization was determined by clearing zone produced around the inoculated spot. The isolate Azt-sut2 and Azt-lko2 showed high level of phosphate solubilization while Azt-uno1& 2, Azt-mth2 andAzt-gnd2 was medium phosphate sulubilizer and Azt-fzb2, Azt-sut1, Azt-gnd1& Azt-knp1recorded with low phosphate solubilization activity. Joshi *et al.* (2011) found that *Azotobacter* isolates were able to solubilize phosphate in the medium. Similarly phosphate solubilization was detected in 74.47% isolates of *Azotobacter* of wheat rhizosphere as reported by Ahmad *et al.* (2008). All isolates could tolerate 8% NaCl concentration and Azt-sut-2, Azt-gnd-1, Azt-gnd-2, Azt-uno-2 and Azt-mth-2 isolates showed their tolerance even at 10% NaCl (Table 2). Rangarajan *et al.* (2002) screened 256 bacterial strains for salt tolerance and 36 strains were able to grow at 6% NaCl while Chaudhay and his coworkers in 2013 reported 6 to 8% NaCl tolerance in *Azotobacter* of wheat rhizosphere.

Table 2: Determination of salt tolerance in *Azotobacter* isolates

Name of isolate	3% NaCl	4% NaCl	5% NaCl	6% NaCl	7% NaCl	8% NaCl	9% NaCl	10% NaCl
Azt-fzb-1	+++	+++	+++	++	+	+	-	-
Azt-fzb-2	+++	+++	+++	+++	+++	++	-	-
Azt--sut-1	+++	+++	+++	+++	+++	++	-	-
Azt--sut-2	+++	+++	+++	+++	+++	+++	++	+
Azt -gnd-1	+++	+++	+++	++	++	+	+	+
Azt -gnd-2	+++	+++	+++	++	++	+	+	+
Azt -bbk-1	+++	+++	+++	++	++	+	-	-
Azt -bbk-2	+++	+++	+++	++	++	+	-	-
Azt -lko-1	+++	+++	+++	+++	+++	+	-	-
Azt -lko-2	+++	+++	+++	+++	+++	++	+	-
Azt -uno-1	+++	+++	+++	+++	+++	++	+	-
Azt -uno-2	+++	+++	+++	+++	+++	+++	+++	++
Azt -knp-1	+++	+++	+++	++	++	+	-	-
Azt -knp-2	+++	+++	+++	++	++	+	-	-
Azt -mth-1	+++	+++	+++	+++	+++	++	+	-
Azt -mth-2	+++	+++	+++	+++	+++	+++	++	++

- = No growth, + = Poor growth, ++= Medium growth, +++ = High growth

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