SHORT COMMUNICATION

QUALITATIVE ASSESSMENT OF *PSEUDOMONAS* ISOLATES ASSOCIATED WITH WHEAT RHIZOSPHERE FOR PHOSPHATE SOLUBILIZING ACTIVITY AND SALT TOLERANCE

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Abstract: Plant growth promoting rhizobacteria (PGPR) are known to influence plant growth by various direct or indirect mechanisms. The plant growth promoting attributes *viz*. production of indole-3-acetic acid (IAA), gibberellins, siderophore, and phosphorus solubilization etc. ability of the rhizobacteria are the most common. The Phosphorus Solubilizing bacteria are used as plant growth promoting bacteria (PGPB). In search of phosphorus solubilizing *Pseudomonas* associated with wheat plants grown in various locations of Uttar Pradesh, we have isolated a total of sixteen strains on the kings'B medium and identified as *Pseudomonas* spp. .Phosphorus solubilizing capabilities as demonstrated by the formation of clearing zone on the pikovaskya medium. Out of 16 *Pseudomonas* strains, only 9strains were found able to solubilize phosphorus. All the *Pseudomonas* strains were screened for salt tolerance. Most of the *Pseudomonas* strains shown tolerance up to 8% NaCl concentration. Only 3 *Pseudomonas* strains were able to grow even at 10% NaCl concentration.

Keywords: Phosphate, Pseudumonas, Rhizobacteria, Wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major cereal crops in India. The wheat crop is mainly cultivated under rainfed condition where precipitation is less than 900mm 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 plant growth promoting attributes *viz*. production of indole-3-acetic acid (IAA), gibberellins, siderophore, and phospate solubilisation etc. ability of the rhizobacteria of wheat in saline area are inexpensive, simple to use and have no adverse effect on land (Upadhyay *et al*., 2009). Phosphorus is an important for plant growth and development (Reddy and Singh, 2011). P 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 (Altomare et al., 1999). This 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).Where inorganic and organic P forms are abundant in soils, their concentration in the soil solution is usually in the micro molar ranges. It has been reported that plant growth promoting rhizobacteria (PGPR) including phosphate-solubilizing microorganisms (PSMs) are able to solubilise the unavailable forms of P in soil by acidification, chelation, and exchange reaction in the soil environment (Maliha *et.al* 2004, Ponmurugan and Gopi, 2006). 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, Rhizobium, 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 *Zea mays* (Saharan and Nehra, 2011). It has been reported that *Pseudomonas* sp. BR2, promotes wheat roots mycorrhization and phosphorus acquisition by wheat plants (Babana *et al*., 2012). Nitrogen fixing and phosphate-solubilizing bacteria are the two main groups biofertilizer of plant growth-promoting bacteria will help evaluate biofertilizer better, and these bacteria would be used on crops to allow reduced rate of fertilizers. In our study, an attempt made to aim characterize the P-solubilizing activitis of rhizobacteria viz. *Pseudomonas* sp. of wheat rhizosphere grown under saline infested zone.

MATERIAL AND METHOD

Isolation of *Pseudomonas* isolates from wheat rhizosphere

Soil and root samples of wheat were collected in sterile plastic bags from eight different salt affected district viz. Faizabad, Sultanpur, Gonda, Barabanki, Lucknow, Unnao, Kanpur Dehat and Mathura in Uttar Pradesh. Samples were collected at flowering stage of plant growth. Rhizospheric soil was separated from roots of wheat with the help of brush in a petriplate. One gram soil of each eight samples 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 Kings 'B' medium with pH 7.0. The plates incubated at 30 ^{0}C for 24 hours.

Biochemical characterization and identification of rhizospheric strains

The strains were identified by physiological, biochemical methods. The physiological and biochemical identification was performed according to Bergey's manual of Systematic Bacteriology (Claus and Berkeley, 1986).

Phosphate solubilization

Each *Pseudomonas* isolate was spot inoculated on Pikovskaya's agar medium (Pikovskaya, 1948) and incubated at 28 ^{0}C up to 5 days. Phosphate solubilization activity was determined by development of clearing zone around inoculated spot. Isolates exhibiting clearing zone on Pikovaskya's agar plate were considered as positive (Table-1). (Wahyudi *et al.,* 2011).

Salt tolerance

Pure cultures of all *Pseudomonas* 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 30 ^{0}C for overnight and observed for presence and absence of growth.(Table-2).

RESULT AND DISCUSSION

Isolation and Biochemical characterization of *Pseudomonas* from rice rhizosphere:

Eight soil samples were collected from rhizosphere of wheat grown in different district of Uttar Pradesh. In this study, a total 14 *Pseudomonas* strains were have isolated from the rhizosphere of wheat. The isolates were characterized on the basis of physiological, biochemical, morphological features, Isolates of *Pseudomonas* species from rhizosphere of different crops were widely studied by valverde *et al.,* 2003, Ahmad *et.al* 2008. Rawat *et.al* 2011,

Phosphate solubilization and Salt tolerance

Only nine *Pseudomonas* 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. Sachdeva *et.al* 2009, Shahab *et.al* 2009 demonstrated that *Pseudomonas* were able to increase the availability of phosphorus in soil. Stress tolerance of PSB strains isolated from saline soil have been reported earlier (Joshi *et al.,* 2011). Certain PSB strains tested for their phosphorus solubilizing ability in the presence of varying NaCl concentration (Saran *et al.,* 2008). All isolates could sustain at 7% NaCl concentration and Ps-fzb-1 Ps-mth-1 Ps-mth-2 isolates shown resistance even at 10% NaCl. (Table-2),Rangarajan *et al.* (2002) screened the bacterial strains for salt tolerance and found 36 strains out of 256 were able to grow at 6% NaCl. Our research findings are well match with earlier reports.

Table 1. Screening of *Pseudomonas* isolates for Phosphorus solubilisation

SN	Name of isolate	High Solubilisation	Medium Solubilisation	Poor Solubilisation	No Solubilisation
1	Ps-fzb-1				-
2	Ps-sut-1	+++			
3	Ps-sut-2				-
4	Ps-sut-3	+++			
5	Ps-gnd-1			+	
6	Ps-gnd-2				-
7	Ps-bbk-1			+	
8	Ps-bbk-2				-
9	Ps-lko-1		++		
10	Ps-uno-1	+++			
11	Ps-knp-1		++		
12	Ps-knp-2				-
13	Ps-mth-1		++		
14	Ps-mth-2	+++			

Table 2. Determination of salt tolerance of *Pseudomonas* isolates from wheat rhizosphere

SN.	Name of isolate	3% NaCl	4% NaCl	5% NaCl	6% NaCl	7% NaCl	8% NaCl	9% NaCl	10% NaCl
1	Ps-fzb-1	+++	+++	+++	+++	+++	++	++	+
2	Ps-sut-1	+++	+++	+++	+++	++	+	-	-
3	Ps-sut-2	+++	+++	+++	+++	++	+	-	-
4	Ps-sut-3	+++	+++	+++	+++	++	+	+	-
5	Ps-gnd-1	+++	+++	+++	+++	+++	++	-	-
6	Ps-gnd-2	+++	+++	+++	++	+	-	-	-
7	Ps-bbk-1	+++	+++	+++	+++	++	+	-	-
8	Ps-bbk-2	+++	+++	+++	+++	+++	++	++	-
9	Ps-lko-1	+++	+++	+++	+++	+++	++	+	-
10	Ps-uno-1	+++	+++	+++	+++	++	+	-	-
11	Ps-knp-1	+++	+++	+++	+++	+++	++	+	-
12	Ps-knp-2	+++	+++	+++	+++	+++	++	+	-
13	Ps-mth-1	+++	+++	+++	+++	++	+	+	+
14	Ps-mth-2	+++	+++	+++	+++	+++	++	++	+

- = No growth , **+** = Poor growth , **++** = Medium growth , **+++** = High growth, **++++** = Very high growth

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