

RESEARCH ARTICLE

## Phosphate solubilizing bacteria promote growth and enhance nutrient uptake by wheat

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### Abstract

Phosphorus (P) fixation limits availability of P to plants in tropical soil, which is a major constraint for crop production. The aim of our research was to isolate, screen and characterize phosphate solubilizing bacteria (PSB) from wheat and evaluate their efficacy in P nutrition in wheat. Upon screening, 9 isolates showing varying level of phosphate solubilizing activity in both agar plate and broth assays using Pikovskaya's medium were obtained. The pH of the culture media was decreased with increased bacterial growth suggesting that they might secrete organic acids to solubilize insoluble phosphorus. *In vitro* wheat seedling bioassay with two superior PSB isolates (PSB1 and PSB8) and varying sources of P revealed that both isolates significantly enhanced seedling growth (shoot and root length, shoot and root dry weight) and nutrient contents (%N, %P and %K) in plant tissue compared to control (no PSB). The performance of PSB8 was superior to PSB1 in respect of all the parameters studied. The PSB8 was tentatively identified as *Pseudomonas* sp. through 16S rRNA gene sequencing. Our results suggest that *Pseudomonas* sp. PSB8 isolated from wheat might be useful for improving P nutrition in wheat in soils with low available P.

**Keywords:** Phosphate solubilizing bacteria; wheat; P nutrition; *Pseudomonas* sp.

### Introduction

Phosphorus (P) is one of the essential mineral macronutrients, which is required for maximizing the yield of crops (Griffith, 2009). In soils, P may exist in many different forms, which can be thought of existing in 3 "pools": solution P, active P and fixed P (Busman, Nalepa, & Dobryniowska, 2009). Generally, a major portion of soil P remains as insoluble forms with cations ( $Al^{3+}$  and  $Fe^{3+}$  in acidic soils, and  $Ca^{2+}$  in calcareous soils), which are usually unavailable for uptake by crop plants (Abd-Alla, 1994; Yadav & Dadarwal, 1997). It has been assumed that, global crop yield up to 30-40% of arable land is limited by low P availability (Von Uexkull & Mutert, 1995). In Bangladesh, a greater portion of soil contains low to medium amounts of available P. A significant reduction in the use of phosphatic fertilizer could be achieved if in some way, solubilization of soil insoluble P is made available to crop plants (Rodriguez & Fraga, 1999; Vessey, 2003; Thakuria *et al.*, 2004; Islam *et al.*, 2007; Islam & Hossain, 2012; Sarker, Islam, Biswas, Alam, & Talukder, 2012). Some bacterial species can mineralize and solubilize soil organic and inorganic P (Hilda & Fraga, 2000; Khiari & Parent, 2005; Islam *et al.*, 2007; Islam & Hossain, 2012; Sarker *et al.*, 2012).

Application of phosphate solubilizing bacteria (PSB) as bioinoculants can solubilize the fixed soil P and applied phosphates resulting in higher crop yields (Gull, Hafeez, Saleem, & Malik, 2004). Therefore, PSB are critical for the transfer of P from poorly available soil pools to plant available forms and are important for maintaining P in readily available pools of soil (Fankem *et al.*, 2006). Seed or soil inoculation with PSB has been known to improve solubilization of fixed soil P and applied phosphates resulting in higher crop yields (Abd-Alla, 1994; Jones & Darrah, 1994; Yadav & Dadarwal, 1997). Several lines of evidence suggest that application of PSB improves plant P nutrition and increases the yield of cereals including wheat (Afzal & Asghari, 2008; Ashrafuzzaman *et al.*, 2009; Islam

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& Hossain 2012). Therefore, isolation and characterization of PSB from wheat plants grown in acidic soils are considered as interesting research target for discovering novel elite strains of PSB (Islam & Hossain, 2012). Scant information is available regarding isolation, and identification of PSB from the rhizosphere of wheat and their application for P nutrition in wheat in the acidic soils of Bangladesh. However, some successful attempts have been made to isolate, screen and characterize PSB from rhizosphere of different crop plants and test their efficacy in crops grown in the acidic soils of Bangladesh (Rahman, Talukder, & Islam, 2005; Islam *et al.*, 2007; Alam, Talukder, Islam, & Rahman, 2008; Sarker *et al.*, 2012). The objectives of current study were to isolate, screen and characterize PSB from the rhizosphere of wheat; evaluate phosphate solubilizing capacity of the isolated PSB and their growth at different pH on culture medium, and assess performance of some superior PSB on growth and nutrient contents in wheat seedlings.

## Materials and methods

### *Preparation of root samples and isolation of rhizosphere bacteria*

The root samples were collected from the 20 day-old wheat seedlings grown at the Analytical laboratory of Department of Agricultural Chemistry using sterile forceps and needles. The roots were cut into small pieces and homogenized by crushing followed by vigorous shaking by a vortex mixture for 1 min in 20 mL distilled water in a sterile test tube. Homogenate was diluted 100-fold by a dilution series down to  $1 \times 10^{-6}$ . Pikovskaya's (PVK) medium was used for isolating possible potential PSB (Pikovskaya, 1948). Exactly 100  $\mu$ L aliquot of each sample (*ca.*  $1 \times 10^{-6}$  dilution series) were taken and spread over the PVK medium for identification of watery clear halo zone of the bacterial strains. From the mixtures of different bacterial strains, the potential PSB were detected and isolated on the basis of clear halo zone (Islam & Hossain, 2012). All inoculated plates were incubated at 25°C for 2 days and observation was continued for 4 days.

### *Screening of phosphate solubilizing bacteria on agar assay*

Mineral phosphate solubilization activities of isolated bacterial strains were tested by plate assay qualitatively (Islam *et al.*, 2007). Briefly, a colony of bacterium was taken from each isolate using sterile toothpicks and inoculating loop and plated onto PVK agar medium containing tricalcium phosphate, making a groove on the medium and incubated at 25°C for 72 h. The halo zone of solubilized P and colony diameters were measured after 7 days of incubation of plates at 25°C. Phosphate solubilizing capacity was calculated in terms of phosphate solubilization index (PSI). The isolates showing  $PSI > 2$  have been considered as PSB (Sarker *et al.*, 2012). The

ratio of total diameter (colony + halo zone) and the colony diameter was measured as PSI (Edi Premono, Moawad, & Vlek, 1996).

### *Quantitative estimation of phosphate solubilization in broth assay*

The quantitative bioassay was carried out using Erlenmeyer flasks (100 mL) containing 50 mL PVK broth inoculated using bacterial isolates with approximately  $1 \times 10^8$ - $10^9$  Colony Forming Units (CFU) per mL. The bacteria were inoculated in the medium having pH 7.0, which was adjusted before autoclaving the medium. The flasks were incubated at 25°C in a shaker for 48 h at 100 rpm. The cultures were collected for centrifugation for 10 min at 5500 rpm. The supernatant was decanted and filtered through Whatman No. 41 filter paper (Islam *et al.*, 2007). The available P content in the supernatant was estimated by phospho-molybdate blue complex colorimetric method at 660 nm wavelength (Olsen, Col, Watanable, & Dean, 1954, Alam *et al.*, 2008). Each treatment was replicated three times and data were expressed as the mean value  $\pm$  standard error (SE).

### *Bacterial growth and pH value of the culture medium*

To analyze whether bacteria can grow in a range of pH 5.5 to 8.5 and to test their ability to change the pH value of the medium, the most superior five strains of PSB were inoculated separately in test tubes (18  $\times$  1.6 cm) containing 50 mL of PVK broth at varying pH levels. Bacteria were grown in a shaking incubator (100 rpm) for 8 days at 25°C. The optical density of the bacterial supernatant after precipitation of insoluble tri-calcium phosphates and pH value of the medium were estimated after 2 days intervals using a spectrophotometer (ALPO, Germany) at 595 nm and a pH meter (Horiba, B-212, Kyoto, Japan), respectively (Islam *et al.*, 2007). Each treatment was replicated three times and data were expressed as the mean value.

### *Performance evaluation of superior bacterial strains through wheat seedling bioassay*

The 500 mL Erlenmeyer flasks were selected for this *in vivo* culture of the wheat seedlings. Two strains PSB1 (PSI 3.936 and P solubilization in broth assay 66.32 mg/L) and PSB8 (PSI 4.08 and P solubilization in broth assay 61.78 mg/L) were selected for this study. The control treatment (no PSB) was designated as PSB0. They were cultured in PVK broth medium for 3 days at  $25 \pm 1^\circ\text{C}$  in a shaker at 100 rpm. When growth was found optimum; the cultures were checked for purity and population. Then these were centrifuged at 5500 rpm repeatedly with deionized distilled water for three times. Then these bacterial cells without any culture media were suspended in sterilized water. These bacterial suspensions were used for seed inoculation of wheat for *in vivo* culture. The selected

variety of wheat was Shatabdi. Seeds were surface sterilized by using 20% Clorox for 1 min and then washed by distilled water for three times. The seeds were then soaked in the bacterial suspension (*ca.*  $1 \times 10^{10}$ ) for 3 h (Alam *et al.*, 2008).

Subsequently, these bacteria-coated seeds were kept in blotting paper for absorbing extra aqueous mass from the seeds and then used for *in vivo* culture on agar (1%) containing Hoagland's media in 500 mL Erlenmeyer flasks for 20 days. Different sources of P used in the study were designated as P<sub>0</sub> = No P fertilization; P<sub>1</sub> = 0.5% P as Triple superphosphate (TSP); P<sub>2</sub> = 0.5% P as Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> and P<sub>3</sub> = 0.5% P as AlPO<sub>4</sub>. After 20 days, the seedlings were harvested and separated into shoot and root for analyses of different growth parameters and nutrient contents in plant tissues. Each treatment was replicated for three times.

#### Determination of nutrient content in plant tissues

The estimation of total nitrogen was made by the semi-micro Kjeldahl method (Page, Miller, & Keeney, 1982). The P content of plant extract was determined colorimetrically by stannous chloride method (Olsen *et al.*, 1954) and potassium was estimated by flame emission spectrophotometer (Page *et al.*, 1982).

#### Molecular identification of active bacteria

For the determination of 16S rRNA sequence of PSB8, chromosomal DNA extraction was done using the commercial DNA extraction kit (ATM TM Genomic DNA Extraction Kit) and quantified using lambda DNA marker after agarose gel electrophoresis. The 16S rRNA region was amplified by PCR using 27F and 1492R universal primer. Thermal cycling was performed with Mastercycler® Gradient (Eppendorf, Hamburg, Germany). PCR direct-sequencing was done using Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA). Labeled PCR-direct mixture was purified and analyzed by sequencer (ABI Prism® 310 Genetic Analyzer, Applied Biosystems) according to the

manufacturer's instructions. Forward and reverse sequences were combined using the Lasergene version 7.1 programs. The 16S rRNA gene sequence data of the PSB8 were subjected to BLASTN search using NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) for DNA-DNA homology study.

#### Experimental design and data analysis

The *in vivo* experiment was laid out in Randomized Complete Block Design (RCBD) having two factors (both factor with similar preference) with 3 replications. The statistical analysis of the experiment was carried out using statistical computer package MSstat-C and PLABstat. The ANOVA followed by Duncan's Multiple Range Test (DMRT) was used to analyze the data.

## Results

### Isolation, screening, and phosphate solubilization by PSB

Nine bacterial isolates (PSB1-9) were isolated from the rhizoplane of wheat and purified by repeated streak culture on NBA medium. To screen whether these bacterial isolates can solubilize phosphates from tricalcium phosphate, PVK agar medium was used in agar plate assay. The phosphate solubilisation index (PSI) of tested bacterial strains ranged from 2.14 to 4.08 (Table 1). Among the tested strains, PSB8 and PSB2 displayed maximum and minimum phosphate solubilization in agar medium, respectively.

The PSB isolates also showed varying level of phosphate solubilization in broth assay. It revealed that the trend of phosphate solubilization by PSB isolates in both agar and broth assays was more or less consistent. The highest phosphate solubilization was recorded in PSB1 (66.32 mg/L) followed by PSB8 (61.78 mg/L) (Table 1). The lowest performance was shown by PSB7 (28.35 mg/L). Considering the superior performances in both agar and broth assays, PSB1 and PSB8 were chosen for further evaluation of their effects on growth and nutrient uptake by wheat *cv.* Shatabdi in seedling assay.

**Table 1. Phosphate solubilizing index and amount of phosphate solubilization by different bacterial isolates in agar and broth assay using Pikovskaya's medium**

Bacterial isolates	PSI* in agar assay**	PSI in broth assay (mg/L)**
PSB1	3.94 ± 0.02	66.32 ± 3.5
PSB2	2.14 ± 0.01	38.36 ± 1.8
PSB3	2.69 ± 0.01	41.29 ± 2.6
PSB4	3.13 ± 0.02	48.58 ± 2.5
PSB5	3.53 ± 0.02	51.59 ± 3.2
PSB6	2.66 ± 0.01	39.06 ± 2.4
PSB7	2.18 ± 0.01	28.65 ± 1.5
PSB8	4.08 ± 0.02	61.78 ± 5.8
PSB9	3.34 ± 0.01	54.32 ± 4.3

\* Phosphate solubilization index (PSI) = (Halo + colony diameter) / colony diameter

\*\*Mean ± Standard Error (SE)

**Table 2. Interaction effect of PSB inoculation and different sources of P on shoot length and root length of wheat seedlings at different days after inoculation (DAI)**

PSB inoculum × Phosphorus interaction	Shoot length (cm)				Root length (cm)
	5 DAI	10 DAI	15 DAI	20 DAI (harvest)	20 DAI (harvest)
PSB <sub>0</sub> P <sub>0</sub>	2.6e ± 0.01	3.6i ± 0.02	5.8f ± 0.01	6.5h ± 0.04	4.6d ± 0.03
PSB <sub>0</sub> P <sub>1</sub>	2.7de ± 0.02	4.1g ± 0.04	6.2e ± 0.04	7.4f ± 0.02	4.2e ± 0.01
PSB <sub>0</sub> P <sub>2</sub>	2.9d ± 0.01	3.8h ± 0.01	6.0e ± 0.02	7.1g ± 0.01	4.7d ± 0.04
PSB <sub>0</sub> P <sub>3</sub>	2.6e ± 0.05	3.8h ± 0.01	5.9f ± 0.01	7.1g ± 0.05	4.8d ± 0.01
PSB <sub>1</sub> P <sub>0</sub>	2.8d ± 0.04	4.5ef ± 0.03	6.7d ± 0.03	7.8f ± 0.01	5.1d ± 0.05
PSB <sub>1</sub> P <sub>1</sub>	3.4b ± 0.03	4.8bc ± 0.03	7.2b ± 0.05	9.4c ± 0.03	6.2b ± 0.03
PSB <sub>1</sub> P <sub>2</sub>	3.2bc ± 0.06	4.6de ± 0.02	7.0c ± 0.04	8.3de ± 0.04	5.7c ± 0.06
PSB <sub>1</sub> P <sub>3</sub>	3.1c ± 0.03	4.7cd ± 0.06	6.9c ± 0.06	8.5d ± 0.05	5.6c ± 0.05
PSB <sub>8</sub> P <sub>0</sub>	2.9d ± 0.05	4.4f ± 0.02	6.6d ± 0.01	8.1e ± 0.02	5.7c ± 0.03
PSB <sub>8</sub> P <sub>1</sub>	3.6a ± 0.02	5.1a ± 0.05	7.8a ± 0.06	10.5a ± 0.06	6.9a ± 0.01
PSB <sub>8</sub> P <sub>2</sub>	3.4b ± 0.06	4.9b ± 0.03	7.6a ± 0.03	9.7b ± 0.06	6.7a ± 0.06
PSB <sub>8</sub> P <sub>3</sub>	3.2c ± 0.04	4.8bc ± 0.06	7.3b ± 0.05	9.3c ± 0.03	6.7a ± 0.06
	**	**	**	**	**

The figures in the column are the mean value of 3 replicates ± Standard Error (SE)

\*\* Significant at p<0.01. Figures in a column followed by same letter(s) are not varied significantly (p<0.05)

PSB<sub>0</sub> = no inoculation of seeds with bacteria; P<sub>0</sub> = no P fertilization; P<sub>1</sub> = 0.5% P as TSP; P<sub>2</sub> = 0.5% P as Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>; and P<sub>3</sub> = 0.5% P as AlPO<sub>4</sub>.

**Table 3. Interaction effect of PSB inoculation and different sources of P on shoot dry weight, root dry weight and nutrient contents in wheat seedlings**

PSB inoculants × levels of P interaction	Shoot dry weight (g)	Root dry weight (g)	Nutrient contents in shoots			Nutrient contents in roots		
			%N	%P	%K	%N	%P	%K
PSB <sub>0</sub> P <sub>0</sub>	0.60e	0.07	0.81g	0.09fg	1.12i	0.50j	0.02i	0.41g
PSB <sub>0</sub> P <sub>1</sub>	0.62e	0.09	0.92e	0.11f	1.16i	0.52i	0.04h	0.46f
PSB <sub>0</sub> P <sub>2</sub>	0.62e	0.10	0.88ef	0.09fg	1.19h	0.61h	0.07g	0.43e
PSB <sub>0</sub> P <sub>3</sub>	0.59e	0.06	0.85fg	0.10f	1.13i	0.72g	0.09f	0.42g
PSB <sub>1</sub> P <sub>0</sub>	0.74bcd	0.12	1.16e	0.21cd	1.67g	1.07f	0.17cd	0.51d
PSB <sub>1</sub> P <sub>1</sub>	0.77ab	0.19	1.43a	0.27b	2.43b	1.22a	0.21a	0.55c
PSB <sub>1</sub> P <sub>2</sub>	0.70d	0.16	1.20d	0.22c	2.13e	1.13d	0.18bc	0.47e
PSB <sub>1</sub> P <sub>3</sub>	0.70cd	0.18	1.19d	0.17e	1.93f	1.16c	0.16d	0.44f
PSB <sub>8</sub> P <sub>0</sub>	0.76abc	0.17	1.32bc	0.29b	2.46b	1.19b	0.14e	0.59b
PSB <sub>8</sub> P <sub>1</sub>	0.81a	0.14	1.36b	0.32a	2.61a	1.18b	0.19b	0.63a
PSB <sub>8</sub> P <sub>2</sub>	0.74bcd	0.15	1.17d	0.20cde	2.34d	1.15c	0.18bc	0.55c
PSB <sub>8</sub> P <sub>3</sub>	0.71cd	0.13	1.29c	0.18de	2.37c	1.11e	0.14e	0.50d
Level of Significance	**	NS	**	**	**	**	*	**

The figures in the column are the mean value of 3 replicates.

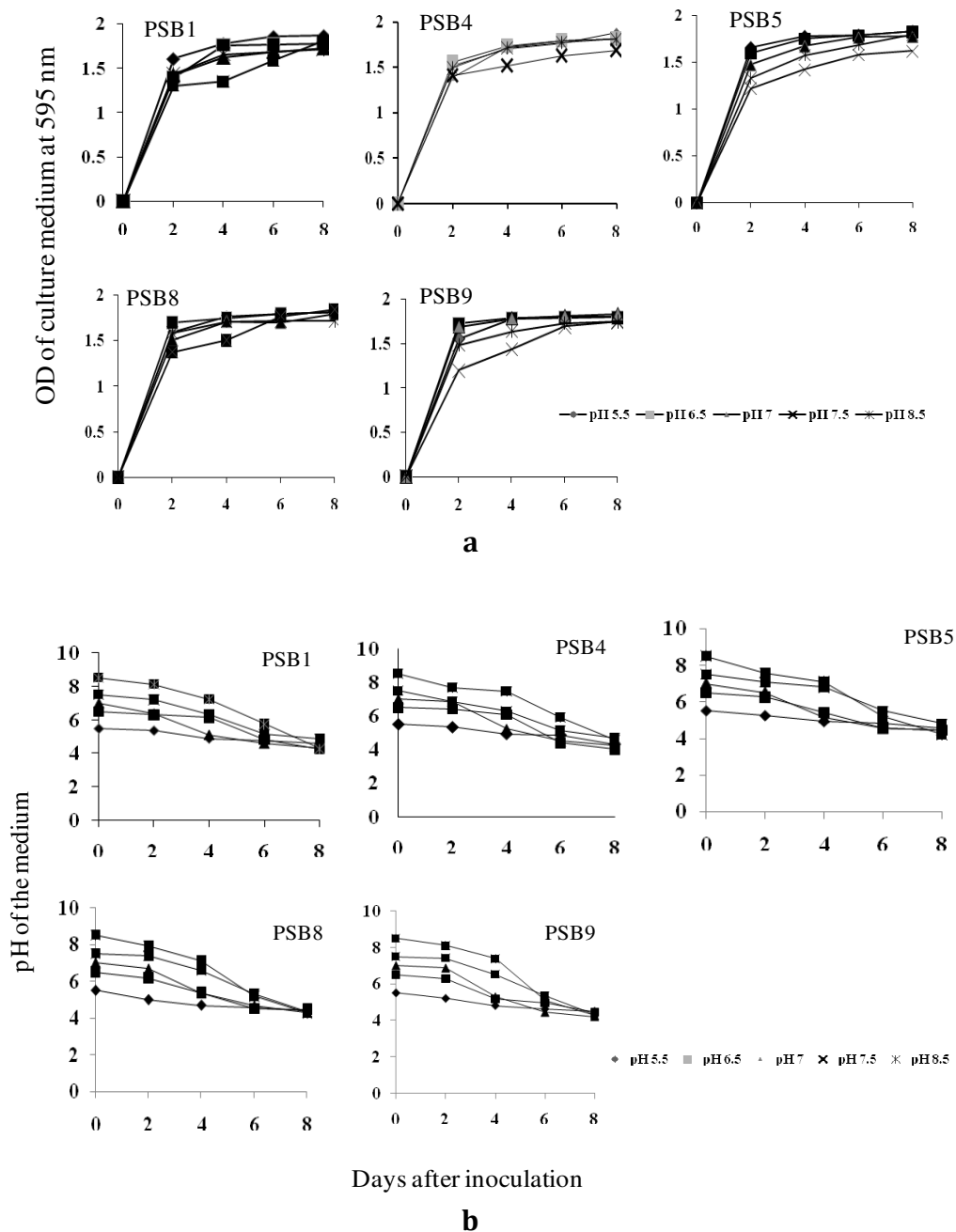
Figures in a column followed by same letter/s are not varied significantly (p<0.05)

\*\* = Significant at p<0.01, \* = Significant at p<0.05. NS = Not significant

#### Growth of the PSB at varying pH

The most superior five PSB isolates were grown in PVK broth medium with varying levels of pH (5.5 to 8.5) up to 8

days to see their growth (optical density, OD values) and the change of the pH values in culture medium. Time-course OD values revealed that the inoculated



**Fig. 1. Changes of the growth of 5 superior PSBs represented by the optical density (a) and the pH value of the culture medium (b) with time of inoculation in Pikovskaya's broth medium**

bacterial strains showed more or less a steady growth on time (Fig. 1a). At pH 5.5, the PSB4 displayed its highest growth (OD, 1.883) followed by the PSB1 (OD, 1.870). Similarly, PSB5 displayed its highest growth (OD, 1.835) at pH 5.5. On the other hand, PSB8 exhibited highest growth (OD, 1.845) at pH 7.5, while PSB9 exhibited its highest growth (OD, 1.832) at pH 7.0.

The pH value of the culture medium of all rhizoplane bacteria was decreased with time at pH ranged from 5.5 to

8.5 (Fig. 1b). These results indicate that the PSB strains are likely to secrete organic acids into the medium to solubilize tricalcium phosphate.

#### *Performance of PSB isolates on growth of wheat seedlings*

**Shoot and root length:** Table 2 shows that shoot lengths varied significantly by the effects of PSB inoculation at different days after inoculation (DAI) (*i.e.* 5, 10, 15 and 20 days). The PSB8 produced the highest shoot length at 5 DAI (2.90 cm) and at harvest (8.10 cm) in seedlings

obtained from seeds previously treated with this bacterium. On the other hand, PSB1 produced the highest shoot length at 10 DAI (4.50 cm) and at 15 DAI (6.70 cm). Other treatments were also influenced the shoot lengths of wheat seedlings in similar manners. As expected, shoot lengths were gradually increased with the age of wheat seedlings irrespective of the treatments. Like shoot lengths, the root lengths also significantly ( $P < 0.05$ ) varied in seedling obtained from seeds treated with both PSB inoculants (PSB1 and PSB8) (Table 2).

**Shoot and root dry weight:** Inoculation of PSB significantly enhanced the shoot dry weight of wheat seedlings compared to uninoculated control (PSB<sub>0</sub>) (Table 3). The treatment PSB8 produced the maximum shoot dry weight (0.76g), which was superior to other treatments (Table 3). Our results showed that there was no significant variation in shoot dry weight due to the effect of different sources of P (Table 3). The root dry weights did not vary significantly due to the effect of inoculation of phosphate solubilizing bacteria. On contrary, there was significant variation in root dry weight due to the effect of different sources of P (Table 3). The treatment P<sub>2</sub> produced highest value of root dry weight (0.10g) (Table 3), which was superior to other treatments.

#### *Effect of PSB inoculation on nutrient contents of wheat tissues*

Contents of nitrogen (N), phosphorus (P), and potassium (K) in shoot and root tissues of wheat seedlings varied significantly upon inoculation of PSB strains (Table 3). The inoculation of PSB8 produced the highest contents of N, P and K in both shoot and root tissues of wheat seedlings (Table 3). On the other hand, the results on the interaction effect of inoculation of PSB and different sources of P showed significant variation (Table 3). The combined treatment of bacterial strain and sources of P (PSB1P1) showed the highest value for %N in shoot and %N and %P in roots, respectively (Table 3). Similarly, the PSB8P<sub>1</sub> treatment produced the highest value for %P and %K for shoot tissues and %K for root tissues. The interaction of bacterial inoculation along with comparatively soluble source of P significantly increased the contents of nutrients in wheat tissues as compared to the other treatment combinations.

#### *Molecular identification of PSB8*

The best performing isolate PSB8 was identified as *Pseudomonas* sp. based on comparison of its 16S rRNA gene sequence data with known bacteria sequences in NCBI database using BLASTN. The 16S rRNA gene sequence data of PSB8 showed only 94% similarity with strains of *Pseudomonas* sp. Therefore, PSB8 seemed to be a new species as its 16S rRNA gene sequence data showed less than 97% similarity to the known *Pseudomonas* sp.

## **Discussion**

In the present study, we isolated 9 PSB from the rhizoplane of wheat. These isolates displayed varying levels of phosphate solubilization in both agar and broth assays probably through secretion of organic acids. Two superior isolates (PSB1 and PSB8) also tested on vegetative growth and nutrient uptake by wheat seedlings. Significant enhanced shoot and root growth and increased nutrient contents in tissues of wheat were recorded when seedlings were grown from seeds previously treated with PSB8 compared to PSB1 and control treatment. The PSB8 tentatively identified as *Pseudomonas* sp. through 16S rRNA gene sequencing. Our results indicate that PSB8 is a potential phosphate solubilizer, which could be utilized as a bioinoculant of P nutrition in wheat in soils under high pH. Solubilizations of insoluble phosphate in calcareous soils and P nutrition in various crops by *Pseudomonas* spp. have been reported by many investigators (Islam & Hossain, 2012). This report identified a potent strain of *Pseudomonas* sp. for the first time from the rhizoplane of wheat grown in Bangladesh.

Our wheat PSB strains displayed consistent performances in solubilizing tricalcium phosphates in both agar and broth culture assays (Table 1). Similar consistent results of phosphate solubilization by PSB were also observed by earlier investigators (Nautiyal, 1999; Islam *et al.*, 2007; Sarker *et al.*, 2012). Optical density of culture medium revealed that the wheat rhizoplane PSB exhibited almost similar growth at pH ranging from 5.5 to 8.5. However, most of the bacterial isolates grew well in slightly acidic conditions (pH 5.5 – pH 6.5). This is reasonable because these bacteria were isolated from the wheat grown in upland soils having pH 5.7. Although, the mechanisms of phosphate solubilization by the bacterial isolates was not clear in our current study, decrease in the pH value of the culture medium recorded by the isolated PSB indicating secretion of organic acids into the medium for tricalcium phosphate solubilization. The secretion of organic acids such as gluconic, 2-ketogluconic, lactic, isovaleric, isobutyric, acetic, oxalic, citric acid etc. by phosphate solubilizing bacteria have been well documented (Rodriguez & Fraga, 1999; Vessey, 2003; Thakuria *et al.*, 2004; Islam & Hossain, 2012).

One of the interesting findings of our study is that seedlings obtained from seeds previously inoculated with PSB8 significantly enhanced shoot and root growth of wheat. Furthermore, not only P but also some other nutrient elements in plant tissues were increased in PSB8 treated wheat seedlings indicating better nutrient uptake by the plants due to bacterial inoculation. The solubilization of P in the rhizosphere is the most common mode of action implicated in plant growth promoting rhizobacteria such as *Pseudomonas* spp. that increases the nutrient availability to host plants (Cattelan, Hartel, &

Fuhrmann, 1999; Richardson, 2001; Islam & Hossain, 2012). Several earlier studies showed that phosphate solubilizing rhizobacteria enhance the growth and yield of many economically important crops (de Freitas, Banerjee, & Germida, 1997; Rodriguez & Fraga, 1999; Nautiyal *et al.*, 2000; Vazquez, Holguin, Puente, Lopez-Cortez, & Bashan, 2000; Islam *et al.*, 2007; Islam & Hossain, 2012).

Another important finding of the current study is that the 16S rRNA gene sequence data of PSB8 displayed only 94% similarity to a plant-associated *Pseudomonas* sp. As less than 97% 16S rRNA gene sequence similarity is indicative of a new species, our potent phosphate solubilizer, PSB8 isolated from wheat seem to be a new species under the genus of *Pseudomonas*. A further molecular-based systematic study including DNA-DNA hybridization and submission of sequence data in the Genbank are needed to confirm the identity of this strain at the species level.

In the current study, we observed that the vegetative growth (shoot and root length, shoot and root dry weights) and nutrient contents of the wheat seedlings were increased significantly after inoculation of seeds with the bacterial isolates. Increased P uptake was also found of both rice and wheat when PSB were applied with rock phosphates (Sharma & Prasad, 2003; Vyas & Gulati, 2009). They also found growth promotion and higher nutrient uptakes in response of PSB inoculation in the cereals. In addition to P solubilization, we can not exclude the possibility of the involvement of nitrogen fixation and secretion of phytohormones by the bacterial inoculant PSB8 for promotion of growth and nutrient uptake by wheat seedlings (Islam & Hossain, 2012).

In conclusion, this study successfully isolated nine PSB from the rhizosphere of wheat grown in slightly acidic soils in Bangladesh. These PSB displayed promising effects in solubilization of phosphate from insoluble tricalcium phosphates in both agar and broth assays. One of the isolate, *Pseudomonas* sp. PSB8 significantly enhanced growth and nutrient uptake by wheat seedlings. This study should be further extended to investigate the potential of PSB8 under field conditions before considering its practical use as an alternative to synthetic superphosphate fertilizer for low input sustainable wheat production.

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