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ISOLATION AND CHARCTERIZATION OF PHOSPHATE SOLUBILIZING BACTERIA FROM TRADITIONAL RICE CULTIVAR OF BANGLADESH AND THEIR EFFECTS ON RICE

M. Z. Surovy[1], D. R. Gupta[1], M. M. Rahman[1], M. A. M. Akanda[2] and M. T. Islam[1*]

Abstract

Plant associated bacteria are promising alternatives to chemical fertilizers for plant growth and yield improvement in an eco-friendly manner. Phosphorus fixation limits availability of P to plants in tropical soil, which is a major problem for crop production. In this study, seeds of two traditional rice cultivars (Lalzira and Darshail) were collected from a Non-Government Organization "Unnoyundhara Bangladesh". A total 6 bacteria were tested for phosphate solubilizing activity. Upon screening, three isolates viz., BTS-8, BTS-9, and BTS-10(B3) showing varying levels of phosphate solubilizing activity in agar plate assay. The pH of the culture media was decreased with increased bacterial growth suggesting that these might secret organic acids to solubilize insoluble phosphorus. *In vitro* rice seedling bioassay with three phosphate solubilizing bacterial (PSB) isolates significantly enhanced seedling growth (shoot and root length, shoot, and root biomass) compared to untreated control. The performance of BTS-10(B3) was superior in respect of all the parameters studied in pot experiment, like shoot biomass (13.23g), root biomass (7.25g), SPAD value (33.15), effective tiller (6.33), 100- grain weight (3.14g), and grain yield per pot (7.82g/pot). The BTS-10(B3) was identified as *Bacillus cereus* by 16S rRNA gene sequencing and might be useful for improving P nutrition in rice soils with low nutrient condition.

Keywords: Phosphate nutrition, *in vitro*, bioassay, 16S rRNA, *Bacillus cereus.*

Introduction

Rice, the staple food and major cereal crop of Bangladesh, dominates Bangladesh agriculture occupying more than 73% of total cropped area. Rice cultivation is the major source of livelihood of the people of Bangladesh and about 82% of the total agricultural production comes in the form of rice (Sarker *et al.,* 2010). To fulfill the demand of increasing population, higher amount of rice production is necessary. Higher crop production demands use of higher amount of fertilizer and pesticides. It was estimated that total fertilizer requirement for rice and other crops in our country was approximately 3.79 million tons in 2010 (Shah *et al.,* 2008). Domestic production covers less than 50% of total demand of nitrogenous fertilizers. Almost all potassium and phosphatic fertilizers and 50% of urea are imported from abroad. To maintain substantial increase in crop yield, farmers need to apply higher doses of fertilizers to the field. Use of these chemical fertilizers is deleterious to the environment and injurious to human and soil health.

[1]Department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, [2]Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.*Corresponding Author: tofazzalislam@yahoo.com.

Phosphorus is essential macro nutrient for plants. Due to rapid immobilization of P fertilizers in the form of Al^{3+} or Fe^{3+} in acidic soils or with Ca^{2+} in calcareous soils (Khan *et al.,* 2017), there is a shortage of available phosphorus for plant nutrition (Merbach *et al.,* 2010). Plant-associated bacteria have high potentials to improve nutrition and protection of plants. A group of beneficial bacteria enhance growth and yield of crop plants via various growth promoting mechanisms are called plant growth promoting bacteria (PGPB). The beneficial effects of these bacteria are delivered through various direct or indirect mechanisms (Chernin and Chet, 2002). Some bacteria species having the capacity to mineralized insoluble phosphorus and they are so called phosphate solubilizing bacteria (PSB). PSB are able to mineralize organic phosphates and solubilize inorganic phosphates via several mechanisms, such as hydrolysis or processes involving enzymes like phosphatases (Oteino *et al.*, 2015, Islam and Hossain, 2012). Phosphatases produce organic and inorganic acids through pH reduction, carbon dioxide formation and the enzymatic reduction of metals (Nautiyal, 1999; Souchie *et al.,* 2005; Barroso and Nahas, 2008; Bashan *et al.*, 2013). In organic acid production mechanisms, gluconic acid (GA) seems to be the most frequent agent of inorganic phosphate solubilization and to a lesser extend α-ketogluconic acid. The organic acids diffuse freely outside the cells releasing high quantities of soluble phosphate from mineral phosphates by supplying both protons and metal complexing organic acid anions (Oteino *et al.*, 2015).

The use of PSB inoculants as biofertilizer provides a promising alternative/amendment to chemical fertilizers (Khan *et al.*, 2017 and Sarker *et al.*, 2014). Therefore, isolation and characterization of PSB from endosphere of rice seeds of Bangladesh and utilizes them as bio-inoculants for growth and grain yield of rice is a new trend of research (Khan *et al.*, 2017). The objectives of current study were to isolate, screen and characterize PSB from endosphere of traditional rice seeds of Bangladesh and evaluate phosphate solubilizing capacity of PSB isolates and assess performance of PSB on growth and yield of rice.

Materials and Methods

Isolation and screening of endophytic bacteria

Traditional rice seed samples (Lalzira and Darshail) were collected from a Non-Government Organization "Unnoyondhara Bangladesh", Zhenaidah, Bangladesh. For isolation of bacteria, approximately 1g of seeds was washed under running tap water and surface sterilized in 5% NaOCl for 1 min. After washing three times with sterilized distilled water (SDW), the seed samples were crushed with a sterilized mortar and pestle. Serial dilutions were prepared from the crushed seeds, and 100 µl aliquots from each dilution of 1×10^{-6}, 1×10^{-7}, and 1×10^{-8} were spread on nutrient broth agar (NBA: Nutrient broth 13.0 g/L and agar 21 g/L) plates and incubated for 2 days at 25 ± 2° C. Morphologically distinct bacterial colonies (on the basis of shape and color of the bacterial colonies) were purified by repeat streak culture on the same medium. The purified isolates were preserved temporarily in 20% glycerol solution at-20º C.

Mineral phosphate solubilizing activities of bacterial isolates was determined by using National Botanical Research Institutes Phosphate (NBRIP) medium (Glucose 10.0g/L; $Ca_3(PO_4)_2$ 5.0g/L; $(NH_4)_2SO_4$ 0.10 g/L; $MgSO_4.7H_2O$ 0.25g/L; KCl 0.20g/L; $MgCl_2.6H_2O$ 5.0g/L; agar 15.0g/L and pH 7.0) (Islam *et al.*, 2007). Each bacterial isolate was inoculated in triplicate on NBRIP agar medium and incubated at 25 ± 2° C for 72 h. The ability of the bacteria to solubilize insoluble tri-calcium phosphate (TCP) was determined by phosphate solubilizing index (PSI) (Sarker *et al.*, 2014).

$$PSI = \frac{A}{B}$$

where A= Total diameter (colony+halo zone) and B= colony diameter

Biochemical characterizations of phosphate solubilizing bacteria

A series of biochemical tests were conducted to characterize the PSB isolates (BTS-8, BTS-9 and BTS-10(B3) using the protocols of Bergey's Manual of Systematic Bacteriology (Bergey *et al.*, 1994). The tests conducted were Gram staining, Catalase test and motility test. For all biochemical tests, bacteria were grown on Nutrient Broth Agar (NBA) medium for three days at 25°C.

Gram staining test

A small drop of bacterial suspension was placed on a clean slide. The drop was spread with a bacterial loop and allowed to dry in the air. The air dried smear was passed once through the top of a spirit lamp and allowed to cool. The smear was stained with crystal violet solution for 1 min, then washed in tap water. The film was immerged into iodine solution for 1 min, washed in tap water and dried. Then ethanol was added on the film and washed it after 1 min in tap water and dried again. Then a counter stain, safranine was added with the film and washed in tap water and dried. The color of the smear was observed under an oil immersion object of a light microscope. The color of the smear appeared violet if the bacterium was Gram positive. On the other hand, the color of the smear was turned red if the bacterium was Gram negative (Cerny, 1976; Conn *et al.*, 1957).

Motility test

The motility of each isolate was tested in sulfide indole motility (SIM) medium. Using a needle, isolates were introduced into test tubes containing SIM, and were incubated at room temperature until the growth was evident (Kirshop and Doyle, 1991). Turbidity away from the line of inoculation was a positive indicator of motility.

Catalase test

Catalase test is done to determine if bacteria possess catalase enzyme. Aerobic and facultative anaerobic bacteria produce hydrogen peroxide as a by-product of aerobic respiratory metabolism. Hydrogen peroxide is highly toxic oxidizing agent. Bacteria are protected from these harmful effects by the enzyme catalase which catalyzes the following reaction (Wheelis, 2008):

$$2H_2O_2 \rightarrow H_2O + O_2$$

A loop-full bacterial culture was taken on a clean slide containing one drop of 3% H_2O_2. Formation of bubbles (release of O_2) from the surface was indicative of positive reaction (Duke and Jarvis, 1972).

Bacterial growth and pH value of the culture medium

The pH value of medium was determined by growing the isolates in NBRIP broth at pH 7.0 in a shaking incubator (100 rpm) for 8 days at 25º C. The optical density of the bacterial supernatant after precipitation of insoluble tri-calcium phosphates and pH value of the medium were estimated after two days intervals using a spectrophotometer (Alpo, Germany) at 595 nm and a pH meter (AD 1000, Adwa, Hungary), respectively (Khan *et al.*, 2017). Each treatment was replicated three times and data were expressed as the mean value.

Performance evaluation of bacterial isolates through rice (cv. BRRI dhan 29) seedling bioassay

The 250 ml Erlenmeyer flasks were selected for this *in vivo* culture of the rice (cv. BRRI dhan29) seedlings. All bacteria were cultured in NB medium for three days at 25º C in a shaker at 100 rpm. When growth was optimum (10^9 CFU/mL), the cultures were checked for purity and population by repeated streak culture on the same medium. Then, bacterial cells were collected via centrifugation at 15,000 rpm for 1 min at 4ºC, and each pellet was washed twice with SDW. The bacterial pellets were re-suspended in SDW, gently shacked and used for seed treatment. Approximately 1g rice seeds were surface sterilized in 5% NaOCl for 1 min and washed three times in SDW. Dry seeds were immersed in each bacterial suspension (10^9 CFU/mL), and the preparation was stirred frequently for five minutes. The treated seeds were spread on a Petri dish and air dried overnight at room temperature. The number of bacterial cells per seed, determined via serial dilutions, was approximately 10^8 CFU/seed (Sarker *et al.* 2012). After seven days, the seedlings were harvested and separated into shoot and root for analyses of growth parameters. Each treatment was replicated for three times.

Experimental site and pot preparation

In order to investigate plant growth promotion ability of PSB isolates, pot experiment was carried out at Department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), *Salna*, Gazipur, Bangladesh. Soil from Salna series in Madhupur tract (AEZ 28) was used. The soil is clayey up to 50 cm depth (Khan *et al.,* 2017) and slightly acidic (pH 6.41) in nature. The used pot soil contained 1.55% organic matter (OM), 0.08% total nitrogen (N), 9 ppm available phosphorus (P) and 0.57 meq 100g-1 soil exchangeable potassium (K) (our unpublished data). Seeds of BRRI dhan29 were collected from the Bangladesh Rice Research Institute (BRRI). Germinated seeds were sown in the well prepared experimental pot at the rate of two seedlings per pot (size 20 × 20 × 30 cm).

Design and treatment of pot experiment

Pot experiment was conducted in a Complete Randomized Design (CRD) with three replications for each treatment. The treatments of the experiment included (i) control; (ii) treated with BTS-8; (iii) treated with BTS-9, and (iv) treated with BTS-10(B3).

Plant growth parameters, such as root and shoot dry weight (g), SPAD value of flag leaf at panicle initiation stage, number of effective tillers, 100 grain weight and total grain weight (g) per pot were recorded.

Bacterial inoculum preparation and application to rice (cv. BRRI dhan 29) plants

PSBs were cultured separately in 250 ml conical flasks containing 200 ml NB broth on an orbital shaker at 100 rpm for 72 h at (25 ± 2) ºC and cells were collected after centrifugation at 15,000 rpm for 1 min at 4º C, followed by two times washing with SDW. For seed coating, seeds were washed thoroughly followed by overnight dipping the seeds into bacterial suspension in SDW (*ca.* 1×10^8 cfu/ml). Freshly harvested bacterial cells were suspended in SDW and then sprayed on rice plants at tillering and flowering stages.

Molecular identification and phylogenetic tree analysis of active bacteria

For the determination of 16S rRNA gene sequence of BTS-10(B3) chromosomal DNA extraction was done using the commercial kit (AccuPrep® Genomic DNA Extraction Kit) using the manufacturers' instructions, stored at -20º C, and used as a template DNA in PCR to amplify the 16S rRNA gene sequencing.16S rRNA region was amplified by using the bacterial-specific primers, 27F (5′- AGAGTTTGATCCTGGCTCAG-3′) and 1492R(5′-GGTTACCTTGTTACGACTT-3′). Thermal cycling was performed with 1X Taq buffer, 0.8 mM dNTP, 1 µl Taq polymerase, 1.0 µl of each primer. Three independent PCR amplifications were performedin a thermo cycler (Mastercycler® Gradient, Eppendorf, Hamburg, Germany) at an annealing temperature of 55º C (40 s), an initial denaturation temperature of 94º C (5 min), 30 amplification cycles with denaturation at 94º C(60 s), annealing (30 s), and extension at 72 ºC (10 min). The PCR product was purified using Quick PCR purification column (Promega, Madison, WI, USA) and sequenced using BigDye terminator cycle sequencing kits (Applied Biosystems, Forster city, CA, USA) by following the manufacturer's instructions. Forward and reverse sequences were combined using the Lasergene version 7.1 programs. The 16S rRNA gene sequences were compared by BLAST search with the sequences available in the GenBank database. The phylogenetic tree was constructed using Phylogeny fr. Software.

Statistical analysis

The data were statistically analyzed using SPSS version 17.0 and Statistix (version 10). Data obtained under various treatments were compared via ANOVA using the least significant differences test (LSD) at 5% ($P \leq 0.05$) probability level of significance.

Results and Discussion

Isolation, screening and phosphate solubilization by PSB

Total six bacterial isolates were isolated from endosphere of two traditional rice cultivars (Lalzira and Darshail) and purified by repeated streak culture on NBA. Among the isolates, three of them viz., BTS-8, BTS-9 (from Lalzira) and BTS-10(B3) (from Darshai) exhibited *in vitro* phosphate solubilization capacity when NBRIP agar medium was used as insoluble phosphate source. Colony shape of all three bacterial isolates was round; BTS-8 was gram positive and rests two were gram negative in nature. BTS-8 showed motility and catalase activity; other two were catalase negative and non-motile. Significant differences were found in the diameters of the phosphate solubilization zone; isolate BTS-10(B3) had the largest average phosphate

solubilization zone (PSI 4.58). Other two isolates BTS-8 and BTS-9 had PSI 3.46 and 2.36 respectively (Table 1, Fig. 1). The solubilization zone occurs due to the presence of some substances, such as organic acids, that are released by microorganisms and solubilize the P in the medium (Islam and Hossain 2012 and Sarker *et al.*, 2014). The presence of these substances generates a translucent zone around the colonies, which is indicative of solubilizing capacity (Souchie *et al.*, 2005). Several studies have investigated the capacity of *Bacillus* sp. and other microorganisms for P solubilization and mineralization in different culture media with varying P sources (Pikovskaya, 1948; *Gupta et al.,* 1994; *Nautiyal,* 1999; *Islam et al.,* 2007; *Ahemad & Khan* 2010, *and Duarah et al.,* 2011). The study of low solubility inorganic P sources, such as tricalcium phosphate (Ca_3PO_4), as inorganic P sources contributes to our understanding of the phosphate solubilizing mechanisms by PSB in both *in vitro* and field conditions (Bashan *et al.*, 2013).

Table 1. Phosphate solubilizing index (PSI) by different bacterial isolates in agar plate assay using NBRIP medium

Bacterial isolates	PSI* in agar assay**
BTS-8	3.46 ± 0.05b
BTS-9	2.36 ± 0.04c
BTS-10(B3)	4.58 ± 0.06a

*Phosphate solubilization index (PSI) = (Halo +colony diameter) / colony dia meter ** Mean value of 3 replicates. Figures in a column followed by same letter/s are not varied significantly ($p<0.05$)

Molecular characterization of BTS-10(B3)

The best performing isolate BTS-10(B3) was identified as *Bacillus cereus* based on comparison of its 16S rRNA gene sequence data with known bacteria sequences in NCBI database using BLASTN. Phylogenetic tree was constructed using other reference probiotic bacteria to know the taxonomic status of strain *Bacillus cereus* BTS-10(B3) (accession number MF489797) isolated from Darshail rice cultivar (Fig. 2). The isolate was affiliated with the members of the genus *Bacillus* and showed 98% similarity with the *Bacillus cereus* ATCC 14579.

Growth of the PSB at pH 7.0

The three PSB isolates were grown in NBRIP broth medium at pH 7.0 up to 8 days to see

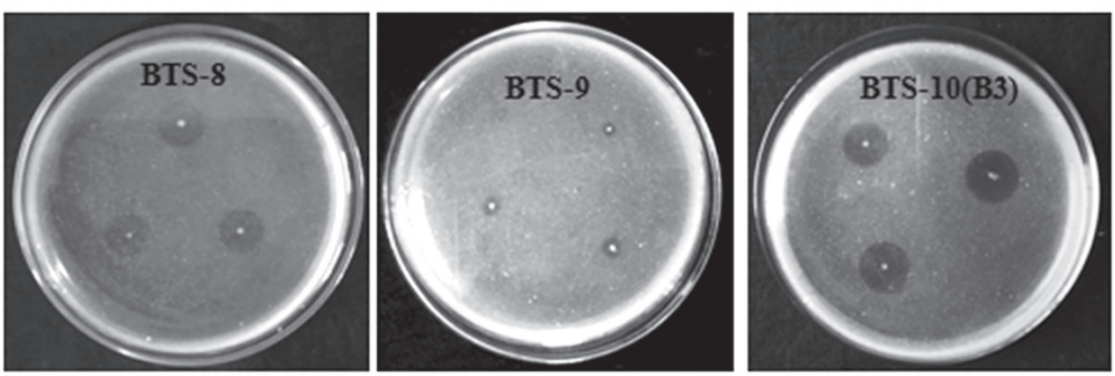

Fig. 1. Characteristic halo zones generated by bacterial isolates around the colonies in the NBRIP medium.

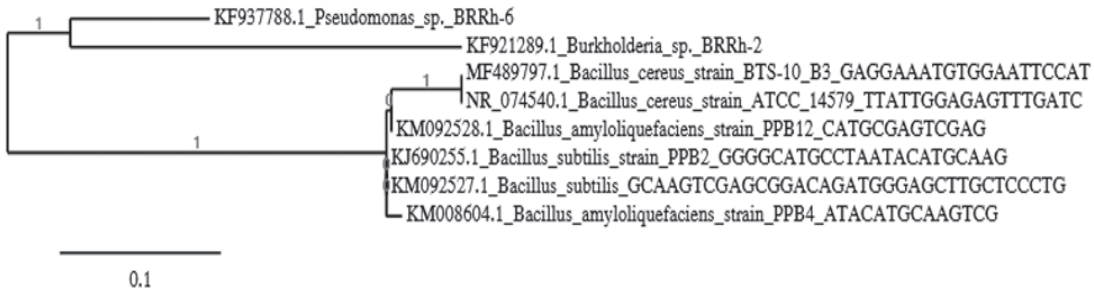

Fig. 2. Phylogenetic tree of *Bacillus cereus* BTS-10(B3) strain using 16S rRNA gene sequence.

their growth (optical density, OD values) and changes of pH value in culture medium. Time- course of OD values revealed that the inoculated bacterial isolates showed more or less a steady growth in course of time (Fig. 3). At eight days after inoculation (DAI) BTS-10(B3) displayed highest growth (OD, 2.23) followed by the BTS-9 (OD, 1.41) and BTS-8 exhibited lowest growth on culture medium (OD, 0.64). The pH value of the culture medium of PSB was decreased with time. These results indicate that the PSB strains a likely to secret organic acids into the medium to solubilize tricalcium phosphate. Acidification of the medium is described as one of the most relevant processes in P solubilization (Bashan *et al.*, 2013). In this process, protons (H) are released. These protons consequently reduce the pH of the medium, leading to the formation of more soluble hydrophosphates (Oteino *et al.* 2015).

Performance of PSB isolates on growth of rice (cv. BRRI dhan 29) seedlings

Shoot and root length

The shoot lengths varied significantly by the effects of PSB inoculation at seven days after inoculation (DAI). The BTS-10(B3) produced the highest shoot length at seven DAI (5.10 cm) in seedlings obtained from seeds previously treated with this bacterium. Other treatments also influenced the growth of rice (cv. BRRI dhan 29) seedlings. Like shoot lengths, the root lengths also significantly ($p<0.05$) varied in seedling obtained from seeds treated with PSB inoculants (Table 2) (Fig. 4).

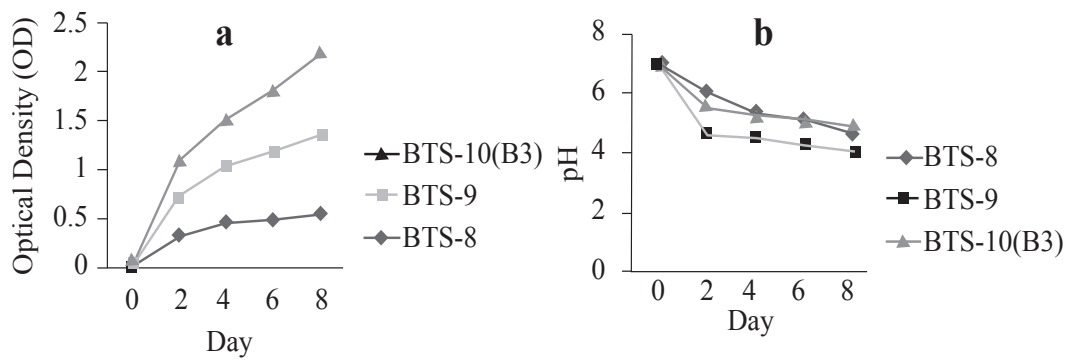

Fig. 3. Changes of the growth of PSBs represented by the optical density (a) and the pH value of the culture medium (b) with time of inoculation in NBRIP medium.

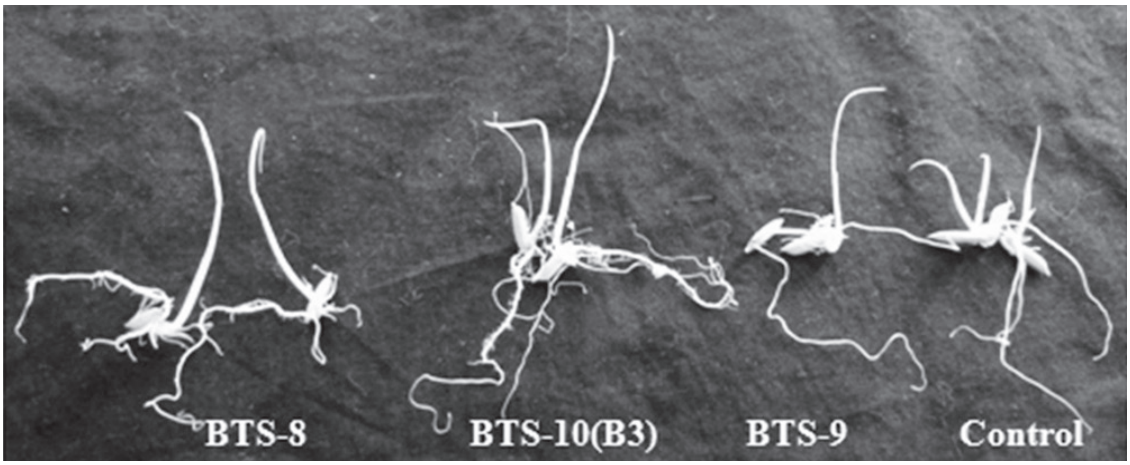

Fig. 4. Effect of endophytic bacteria on root and shoot growth of rice (cv. BRRI dhan 29) seedling at day 7.

Shoot and root biomass

Inoculation of PSB significantly enhanced the shoot biomass of rice (cv. BRRI dhan 29) compared to control (Table 2). The treatment BTS-10(B3) produced the maximum shoot biomass (17.56 mg), which was superior to other treatments (Table 2). The root biomass significantly varied due to the effect of PSB inoculation. The BTS-10(B3) produced highest value of root biomass (13.02 mg) (Table 2), which was superior to other treatments. Increased plant growth and phosphate uptake have been reported in many crop species as a result of PSB inoculants e.g., *Pseudomonas aeruginosa* in rice (Singh *et al.*, 2013), Bacillus sp. in maize (Canbolat *et al.,* 2006) and *B. amyloliquefaciens* in wheat, maize and cotton (Egamberdiyeva *et al.*, 2003). Panhwar *et al.* (2011) showed that the inoculation of rice with *Bacillus* sp. PSB9 isolate enhance P uptake, plant biomass, plant height and root morphology compared to the control.

Effect of PSB inoculation on grain yield of rice (cv. BRRI dhan 29) in pot experiment

The grain yield of rice positively related to SPAD (Soil, Plant Analysis and Development) value. Nitrogen (N) nutrition index is correlated with SPAD value, higher SPAD value indicates higher N nutrition index (Yang *et al.*, 2014). The PSB isolates showed varying levels of SPAD value. The SPAD value was recorded in panicle initiation stage of rice (cv. BRRI dhan 29) plants. SPAD value was ranged from 20.03- 33.15 and highest value was recorded in BTS-10(B3) treated plants (33.15) and the lowest was in control plants (24.03) (Table 3). The highest shoot dry weight and root dry weight was also recorded in BTS-10(B3) treated plants and it was 13.23 g and 7.25 g, respectively. The highest number of effective tillers was also observed in BTS-10(B3) treated plants (Table 3). Similarly, the BTS-10(B3) treatment produced the highest value for 100 grain weight (3.14 g) and grain yield per pot (7.82 g) compared to control (3.04 g and 5.46g, respectively). The interaction of BTS-10(B) bacterial inoculation significantly increased the grain yield of rice (43.22%) as compared to control treatment (Table 3). Hussain *et al.* (2013) found that *Bacillus* sp. significantly increased plant height, root length, shoot dry

Table 2. Interaction effect of PSB inoculation on shoots and root length; shoot and root weight of rice (cv. BRRI dhan 29) seedlings at 7 days after inoculation (DAI)

Bacterial isolates	Shoot			Root		
	Length(cm)	Fresh wt. (mg)	Dry wt. (mg)	Length (cm)	Fresh wt. (mg)	Dry wt. (mg)
BTS-8	4.20±0.08b	11.70±0.80c	0.92±0.01b	5.72±0.18 a	11.00±1.52b	0.26±0.02b
BTS-9	4.10±0.02b	13.76±0.17b	1.02±0.01b	5.33±0.19a	12.36±0.52a	0.31±0.001b
BTS-10 (B3)	5.10±0.56a	17.56±0.96a	1.73±0.09a	5.17±0.06a	13.02±0.50a	0.46±0.03a
Control	4.46±0.04b	10.67±0.02d	0.32±0.04c	3.48±0.20b	10.00±1.14b	0.11±0.10c
CV	6.81	1.56	5.13	6.30	6.06	8.68

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 ($p<0.05$)

Table 3. Effect of PSB inoculation on growth and grain yield of rice (cv. BRRI dhan 29)

Bacterial isolates	SPAD	Shoot dry wt. (g)	Root dry wt. (g)	Effective tiller	100 grain wt. (g)	Grain yield (g/ pot)
BTS-8	30.04±0.03b	12.2±1.04ab	6.83±0.12a	5.00±0.00b	2.91±0.20a	7.51±0.14a
BTS-9	31.05±2.40b	11.15±2.31b	6.32±0.02b	6.00±0.00a	3.02±0.08a	6.50±0.56b
BTS-10(B3)	33.15±1.43a	13.23±0.75a	7.25±0.07a	6.33±0.01a	3.14±0.01a	7.82±0.48a
Control	24.03±0.05c	11.17±0.35b	7.10±0.13a	4.67±0.02b	3.04±0.08a	5.46±0.05c
CV	2.53	6.67	2.66	8.00	7.68	6.20

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 ($p<0.05$)

weight, and root dry weight and grain yield up to 11% over the control. In this current study, rice seedlings treated with PSB inoculant BTS-10(B3) significantly increased shoot and root biomass as well as grain yield of rice by increasing nutrient availability to the host plants. Previously, it has been reported that PSB inoculants not only enhance P availability but also some other nutrient elements in plant tissues (Sarker *et al.*, 2014). Panhwar *et al.* (2011); Islam and Hossain (2012); Sharma and Prasad (2003) and Vyas and Gulati (2009) also observed growth promotion and higher nutrient uptakes in response of PSB inoculation in wheat and rice which supports our findings.

Additionally, possibility of nitrogen fixation and secretion of phytohormones and organic acids, such as gluconic, 2-ketogluconic, lactic, isovaleric, succinic, isobutyric acid, oxalic, citric acid, etc. by PSB inoculants may help in growth promotion and nutrient uptake by rice plants (Chen *et al.*, 2006; Sarker *et al.,* 2014; Islam and Hossain, 2012).

In conclusion, three PSB from endosphere of rice seeds were displayed promising effects in solubilization of insoluble tricalcium phosphate in agar plate assay. *B. cereus* BTS-10(B3) isolate significantly enhanced growth and yield of rice (cv. BRRI dhan29). Current study was conducted in controlled condition, but further study is needed to investigate the efficacy of BTS-10(B3) in broad scale field experiment before considering it as an alternative to synthetic phosphatic fertilizer for low cost rice production.

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References

Ahemed, M and M. S. Khan. 2010. Plant growth promoting activities of phosphate-solubilizing *Enterobacter asburiae* as influenced by fungicides. *EurAsia J. Agric. Biol.* 10: 85-88.

Barroso, C. B. and E. Nahas. 2008. Solubilizacao do fosfato de ferro em meio de cultura. *Pesq. Agropec. Bras.* 43: 529-535.

Bashan, Y., A. A. Kamnev and L. DeBashan. 2013. A proposal for isolating and testing phosphate solubilizing bacteria that enhance plant growth. *Biol. Fertil. Soils.* 49: 1-2.

Bergey, D. H., J. G. Holt and R. K. Noel. 1994. *Bergeys Systematic Bacteriology*. Williams and Wilkins, Baltimore, MD. 1935-2045.

Canbolat, M. Y., S. Bilen, R. Cakmakei, F. Sahin and A. Aydin. 2006. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties, and bacterial counts. *Biol. Fertile. Soils* 42: 350-357.

Cerny, G. 1976. Method for distinction of Gram positive bacteria. *Eur. J. Appl. Microbiol*. 3: 223-225.

Chernin, L. and H. Chet. 2002. Microbial enzymes in the biocontrol of plant pathogens and pests. In *Enzymes in the Environment – Activity, Ecology and Applications.* Burns R G, Dick R P (eds). Marcel Dekker, New York. 171-226.

Chen, Y. P., P. D. Rekha, A. B. Arun, F. T. Shen, W. A. Lai and C. C. Young. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *App. Soil Ecol.* 34: 33-41.

Conn, H. J., W. J. Bartholomew and W. M. Jennison. 1957. *Staining Methods*. McGrew-Hill Book Co. New York. 10- 18.

Duarah, I., M. Deka, N. Saikia and H. P. D. Boruah. 2011. Phosphate solubilizers enhance NPK fertilizer use efiiciency in rice and legume cultivation. Biotech. Doi: 10.1007/s13205-011-0028-2.

Duke, P. B. and J. D. Jarvis. 1972. The catalase test- a cautionary tale. *J. Med. Lab Technol.* 29: 203-204.

Egamberdiyeva, D., D. Juraeva, S. Poberejskaya, O. Myachina, P. Teryuhova, L. Seyadalieva and A. Aliev. 2003. Improvement of wheat and cotton growth and nutrient uptake by phosphate solubilizing bacteria. 26th Southern Conservation Tillage Conference.

Gupta, R., R. Singal, A. Sankar, R. C. Kuhad and R.K. Saxena. 1994. A modified plate assay for screening phosphate solubilizing microorganisms. *J. Gen. Appl. Microbiol*. 40: 255-260.

Hussain, M. I., H. N. Asghar, M. J. Akhtar and Arshad. 2013. Impact of phosphate solubilising bacteria on growth and yield of maize. *Soil Environ*. 32:71–78.

Islam, M. T., A. Deora, Y. Hashidoko, A. Rahman, T. Ito and S. Tahara. 2007. Isolation and identification of potential phosphate solubilizing bacteria from the rhizoplane of *Oryza sativa* L. cv. BR29 of Bangladesh. *Z Naturforsch A*. 62: 103–110.

Islam, M. T. and M. M. Hossain. 2012. Plant probiotics in phosphorus nutrition in crops, with special reference to rice. In: Maheshwari D K. *Bacteria in Agrobiology: Plant Probiotics.* Springer, Berlin Heidelberg: 325–363 P.

Khan, M. M. A., E. Haque, N. C. Paul, M. A. K. Saleh, M.S. Al-Garni and M.T. Islam.

2017. Enhancement of growth and grain yield of BRRI Dhan-29 in nutrient deficient soils by rice probiotic bacteria. *Rice Sci.* 24(5): 264-273.

Krishop, B. E. and A. Doyle. 1991. Maintenance of Microorganisms and Cultural Cells: A Manual of Laboratory Methods. 2nd ed. Academia press, London.

Merbach, W., A. Deubel, A. Gransee, S. Ruppel and A. K. Klamroth. 2010. Phosphorus solubilization in the rhizosphere and its possible importance to determine phosphate plant availability in soil. A review with main emphasis on German results. *Arch. Agron. Sol. Sci*. 56: 119-138.

Nautiyal, C. S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol. Lett.* 170: 265-270.

Oteino, N, R. D. Lally, S. Kiwanuka, A. Lloyd, D. Ryan, K. J. Germaine and D. N. Dowling. 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front. Microbiol.* 6: 745.

Panhwar, Q. A., R. Othman, A. R. Zaharah, M. Sariah and U.A. Naher. 2011. Contribution of phosphate-solubilizing bacteria in phosphorus bioavailability and growth enhancement of aerobic rice. *Span. J. Agric. Res.* 3: 810-820.

Pikovskaya, R. I. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologiya*. 17: 362-370.

Sarker, M. S. K., M. R. Hasan, M. A. Ferdous, M.M.H. Shuhel and Moniruzzaman. 2010. Comparative economic analysis of borrower and non-borrower boro rice farmers' in some selected sites of Mymensingh district. *Bangladesh J. Agril. Res.* 35: 65-76.

Sarker, A., M. T. Islam, G. C. Biswas, M. S. Alam, M. Hossain and N. M. Talukder. 2012. Screening for phosphate solubilizing bacteria inhibiting the rhizoplane of rice grown in acidic soil of Bangladesh. *Acta Microbiologica Immunologica Hungarica*. 59: 199-213.

Sarker, A., N. M. Talukdar and M.T. Islam. Phosphate solubilizing bacteria promote growth and enhance nutrient uptake by wheat. 2014. *Plant Sci. Today.* 1: 86-93.

Shah, A. L., M. S. Rahman and M. A. Aziz. 2008. Outlook for fertilizer consumption and food production in Bangladesh. *Bangladesh J. Agric. Environ*. 4: 9-26.

Sharma, S. N. and R. Prasad. 2003. Yield and P uptake by rice and wheat grown in a sequence as influenced by phosphate fertilization with diammonium phosphate and Mussoorie rock phosphate with or without crop residues and phosphate solubilizing bacteria. *J. Agril. Sci.* 141: 359-369.

Singh, R. K., N. Malik and S. Singh. 2013. Improved nutrient use efficiency increases plant growth of rice with the use of IAA-overproducing strains of endophytic *Burkholderia cepacia* strain RRE25. *Microbial Ecol*. 66: 375-384.

Souchie, E. L., R. Ázcon, J. M. Barea, O. J. Saggin junior and E. M. R. Silva. 2005. Solubilização de fosfatos emn meio sólido e líquido por bactérias e fungos do solo. *Pesq. Agropec. Bras*. 40: 1149-1152.

Vyas, P. P. and A. Gulati. 2009. Stress tolerance and genetic variability of phosphate-solubilizing fluroscent *Pseudomonas* from the cold deserts of the trans-Himalayas. *Microb. Ecol.* 58: 425-434.

Wheelis, M. 2008. *Principles of Mordern Microbiology*. Jones and Barlett Publisher, Inc., Sudbury, MA.

Yang, H., J. Yang, Y. Lv and J. He. 2014. SPAD values and nitrogen nutrition index for the evaluation of rice nitrogen status. *Plant Prod. Sci.* 17: 81-92.