Octa Journal of Environmental Research *Jul. – Sept., 2016*
International Peer-Reviewed Journal ISSN 2321 3655
Oct. Jour. Env. Res. Vol. 4(3): 198-207
Available online http://www.sciencebeingjournal.com

Research Article

Octa Journal of Environmental Research
(Oct. Jour. Env. Res.) ISSN: 2321-3655
Journal Homepage: http://www.sciencebeingjournal.com

ISOLATION AND SCREENING OF POTENTIAL PHOSPHATE SOLUBILIZING BACTERIA (PSB) FROM TIDAL SALINE SOILS OF BANGLADESH

D. Mondal[a], M.S. Islam[a,b]*, M.F. Hoque[a], M.K. Hossain[a], M.K. Islam[a], M.S. Hossin[a] and S.M. Ahsan[c]

a. Deptt. of Soil Science, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh.
b. Graduate School of Environment and Information Sciences, Yokohama National University, 79-7 Tokiwadai, Hodogaya-ku,Yokohama, Kanagawa 240-8501, Japan.
c. Deptt. of Horticulture, Patuakhali Science and Technology University, Dumki, Patuakhali 8602, Bangladesh
*Corresponding author's Email: **islam-md.saiful-nj@ynu.jp**

Received: 19th Aug. 2016 **Revised**: 22th Aug. 2016 **Accepted**: 1st Sept. 2016

Abstract: Phosphorus is one of the major nutrients which play an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant. Many soil bacteria and fungi have the ability to solubilize phosphate minerals and make it available to plants. Thus, an attempt was made to isolate and screen potential phosphate solubilizing bacteria (PSB) which can able to solubilize phosphate and be used as biofertilizer in future. Rice rhizosphere soil samples from the different sites of Khulna and Patuakhali district were collected and each sample was enriched in Pikovskaya's medium, pH 7.5 at 27 ˚C for 5 days. Nine isolates (DMP_1, DMP_2, DMP_3, KNP_4, KNP_5, KNP_6, KKP_7, KKP_8 and KKP_9) were screened using Pikovskaya's liquid medium. All the isolates were oval to rod-shaped, motile and gram positive and gram negative. Biochemical tests indicated that they were obligate aerobes, catalase and starch hydrolysis positive. The phosphate released by the isolate ranged from 4.048 mg/L to 21.128 mg/L. The results revealed that the KNP_4, KKP_7, KNP_5, DMP_3 and DMP_2 isolates had potential to solubilize the insoluble phosphate in the salt affected soil.

Keyword: Biochemical; Biofertilizer; PSB; Rhizosphere.

Postal Address: Department of Soil Science, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh Phone: +88 01717372057

INTRODUCTION

Phosphorus is a biocritical element in short supply in nature. Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant (Gyaneshwar *et al.,* 2002). Soils are often high in insoluble minerals and organic phosphates but deficient in organophosphates (Pi) (Grover, 2003). Approximately 95–99% of soil phosphorous is present in the form of insoluble phosphates and hence cannot be utilized by the plants (Alok *et al.,* 2013). Phosphate anions ($H_2PO_4^-$, HPO_4^{2-}) are extremely reactive and form metal complexes with Ca in calcareous soils (Lindsay *et al.* 1989) and Fe^{3+} and Al^{3+} (Norrish and Rosser, 1983) in acidic soils. These metal ion complexes precipitated the 80% of added P fertilizer and becomes unavailable to plants (Stevenson, 1986; Goldstein, 1986; Yadav and Dadarwal, 1997). Farmers are thus asked to apply phosphorus fertilizers in several-fold excess in order to overcome this problem. In Bangladesh, majority of the phosphorus is provided in the form of chemical fertilizers which abundant use decreases the fertility of soil after long period of time. Due to high cost of mineral superphosphate fertilizers, the resource-poor farmers of Bangladesh often fail to apply

recommended doses of P fertilizers to the soil. Therefore, the release of insoluble and fixed forms of phosphorus is an important aspect of increasing soil phosphorus availability. Plant root-associated phosphate solubilizing bacteria (PSB) have been considered as one of the possible alternatives for inorganic phosphate fertilizers for promoting plant growth and yield (de-Freitas *et al.* 1997; Rodrıguez and Fraga, 1999; Richardson, 2001; Vessey, 2003; Thakuria *et al.* 2004). Due to the negative environmental impacts of chemical fertilizers and their increasing costs, the use of PSB is advantageous in the sustainable agricultural practices.

Phosphate-solubilizing microorganisms play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers. Microorganisms are involved in a range of process that affect the transformation of soil Phosphorus (P) and thus are integral component of the soil 'P' cycle. Several mechanisms like lowering of soil pH by acid production, ion chelation, and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by PSB (Alok *et al.* 2013). Seed or soil inoculation with PSB is known to improve the solubilization of fixed soil phosphorus and applied phosphates, resulting in higher crop yield (Yahya and Al-Azawi, 1989; Abd-Alla, 1994; Mehta and Nautiyal, 2001). Phosphate solubilizing microbes can also produce phosphatase enzyme which can benefit sustainable organic farming systems especially in coastal ecosystems, and reduce the utilization of agrochemicals in agricultural fields (Widawati, 2011).

Total area of Bangladesh 14.4 million hectares (ha) among this coastal area covers about 20% of the country and over 30% percent of the net cultivable area which spreads inside up to 150 km from the coast (Mondal *et al.,* 2016). The diversity of soils and vegetation in Bangladesh is high. Under these conditions, there is a prospect of using PSB inocula in crop production systems. The soils of Bangladesh are highly fertilized with enormous quantities of inorganic phosphate fertilizers (Anonymous, 1997). A significant reduction in the use of phosphate fertilizer could be achieved if solubilization of soil-insoluble phosphorus is made available to crop plants (Rodrı´guez and Fraga, 1999; Vessey, 2003; Thakuria *et al.,* 2004). However, very few attempts have been made to isolate and characterize the potential PSB from the rhizosphere of rice in Bangladesh. Hence, little information is available concerning phosphate solubilizing bacteria and their ability to colonize rice roots in Bangladesh. Thus, an attempt was made to isolate and screen potential phosphate solubilizing bacteria from the soil for the agricultural purposes.

EXPERIMENTAL

Collection and preparation of soil samples: The soil samples used for bacterial isolation were collected from rhizospheric soil fractions and root-free soils from the salinity affected two districts namely Khulna and Patuakhali located at the southern part of Bangladesh (Figure 1) in sterilized pollythene bags. The sampling was conducted in March-April, 2014. At each sampling station, soil sample (up to 20 cm) was collected in the form of sub-samples at a distance of about 20 m each from the first sub-sample in five different locations. These sub-samples were thoroughly mixed to form a composite sample. Then samples were used to investigate on salinity tolerant Rhizobium sp. The tests were conducted at the Central laboratory and Soil Science laboratory of Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh.

Isolation of Phosphate Solubilizing Bacteria (PSB) from rhizosphere soil: Soil samples were prepared by inoculating 1.0 gm of each soil into 100mL of sterile distilled water. Homogenization of soil was carried out by keeping it on shaker for 1hour at 60 rpm at 27°C temperature. After 1hour, samples were removed aseptically, diluted and their dilutions (10-fold dilutions) were plated by the drop plate method on to Pikovskaya's growth medium (10 g/L D-glucose, 5 g/L tricalcium phosphate [$Ca_3(PO_4)_2$], 0.5 g/L $(NH_4)_2SO_4$, 0.2 g/L NaCl,

0.2 g/L KCl, 0.1 g/L $MgSO_4.7H_2O$, 0.5 per g yeast extract, $MnSO_4$ (Traces), $FeSO_4$ (Traces) and 15 g/L agar, pH 7.0) (Pikovskaya, 1948). The plates were incubated aerobically at 30°C for 5 days and an appearance of clear zone around the bacterial colonies was taken as indicator of phosphate solubilization. Colonies with distinct clear zones were re-streaked on Pikovskaya's medium for pure culture isolation. Nine isolates were isolated and assigned as DMP_1, DMP_2, DMP_3, NKP_4, NKP_5, NKP_6, KKP_7, KKP_8 and KKP_9 and slant was prepared as mother culture (Figure 4).

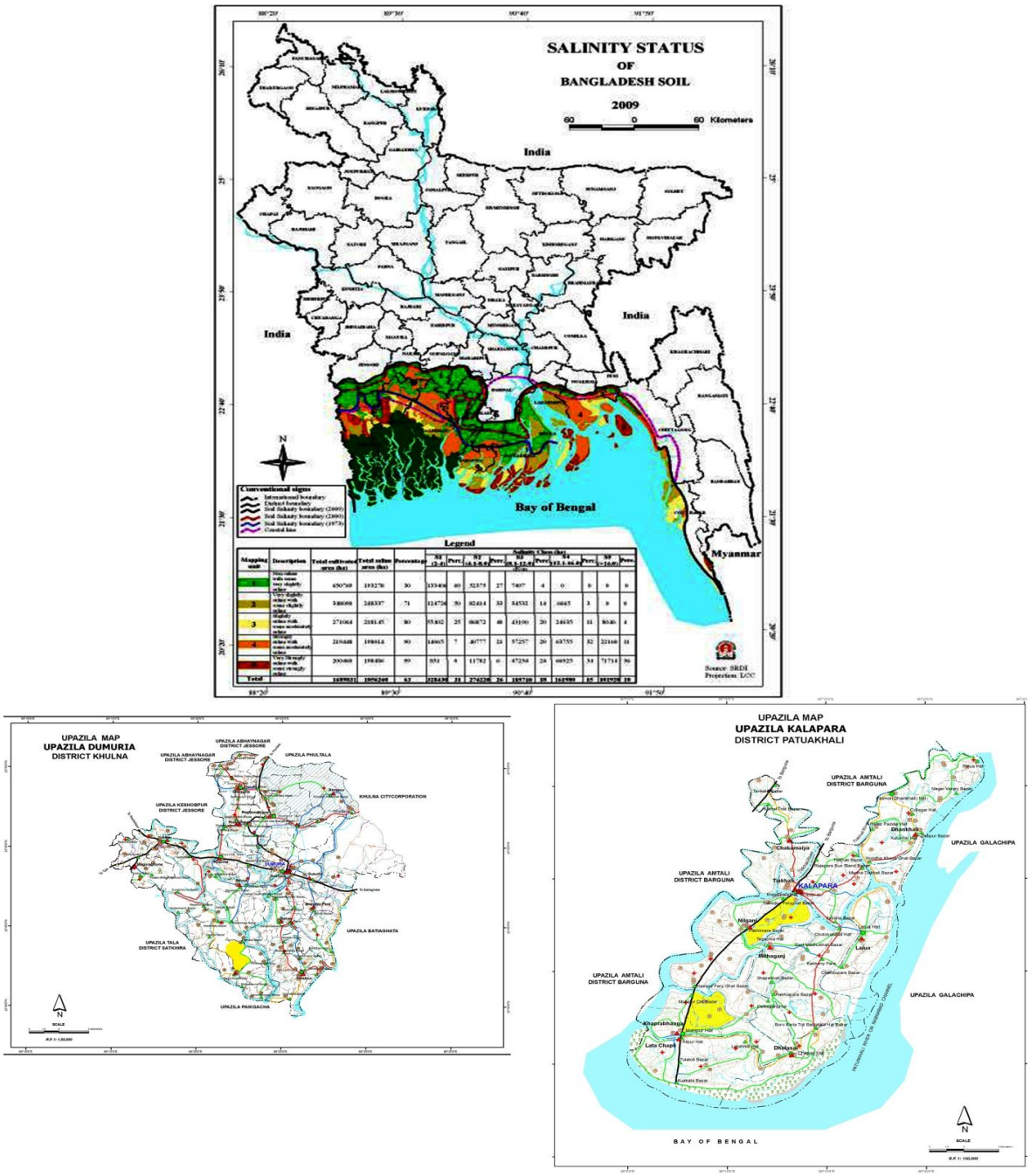


Figure 1. Study area of Khulna and Patuakhali districts located at the southern part of Bangladesh

Physiological Characteristics

Colony morphology: The colony morphology of isolates was examined on Pikovskaya's agar plates. After an incubation of 2-3 days at 30°C, individual colonies were characterized based on their colour, shape, appearance, colony, diameter, transparency and gram stain reaction (Aneja, 2003).

Catalase test: The test was performed to determine the ability of PSB to produce oxygen (O_2) hydrolyzed the 3% of H_2O_2 solution was prepared. One drop of distilled water was taken on clean glass slide. Then colony of 2 days old culture was transferred on water and suspension was made. One drop of H_2O_2 solution was placed on the suspension. Effervescence showed a positive result.

Starch hydrolysis: Starch hydrolysis test was performed to determine the ability of microorganisms to use starch as a carbon source (de Oliverira et. al., 2007). This medium was inoculated with PSB and analyzed for starch utilization. Iodine test was used to determine the capability of microorganisms to use starch. Drops of iodine solution (0.1 N) were spread on a 48 h-old culture grown on Petri plates. Formation of blue color starch iodine complex indicated non utilization of starch and vice versa.

Bromotyhmol blue test: Bromotyhmol blue test was done to identify the isolates as fast grower or slow grower. 5 ml of 0.5% bromothymol blue solution was thoroughly mixed with one liter of Pikovskaya's medium and plated. Culture of different PSB isolates was streaked on the plates and incubated at 30°C and observed.

pH variation assay: The ability of the PSB isolates to grow in basic or acidic media was tested by streaking them on Pikovskaya's agar plates with pH adjusted to 5.0, 6.0, 7.0, 8.0, and 9.0 with HCl or NaOH (Graham *et. al.,* 1991). According to Kucuk *et al.* (2006), the data were noted as (-) = absence of growth, (+) = weak growth and (++) = high growth.

Screening of Potential Phosphate Solubilizing Bacteria

Fifty mL of Pikovskaya's liquid medium containing 25 mg P_2O_5 of phosphorus were poured into 250 ml Erlenmeyer conical flask and sterilized at 15 lb pressure for 15 minutes. The contents of the flask were inoculated in triplicate with 0.5 mL of 48 hours growth of the respective cultures. The un-inoculated control flask in triplicate was also run along with the inoculated flask. All flasks were inoculated at 30°C for seven days on which 2-3 drops of toluene was added to stop the growth. After that volume was made up to 50 ml and the suspension was then centrifuged at 15000 rpm for 30 minutes to remove bacterial cells and other insoluble materials. The pH of the clear liquid was determined with pH meter (HANNA pH 211). The water soluble P_2O_5 in the clear supernatant was estimated by Olsen method (Olsen *et al*., 1954).The analysis of variance was performed and means were compared by Duncan's Multiple Range Test (DMRT) for interpretation of results in MSTAT-C program.

RESULTS AND DISCUSSION

The cells of PSB isolates were examined under light microscope and were found that the cells were oval, shot rod and motile (Table 1). The isolates absorbed counter stain as they were Gram negative bacteria (Figure 5).

Catalase test

Results presented in the Table 1 showed that most of the tested isolates gave positive results but one isolate gave negative results for catalase test. Eight isolates produced bubbles within a few seconds and one (DMP_2) did not produce (Figure 6). Aerobic bacteria hydrolyze hydrogen peroxide (H_2O_2) and produce oxygen (O_2) that makes bubble. Phosphate solubilizing bacteria may be aerobic or anaerobic. So bubbles producing isolates are aerobic and another is anaerobic.

Bromotyhmol blue test

All isolates viz., DMP_1, DMP_2, DMP_3, KNP_4, KNP_5, KNP_6, KKP_7, KKP_8 and KKP_9 developed yellow color (Figure 7) that indicates they are acidic in nature.

Growth in different pH

All isolates grew in PKV medium with pH values of 8, but differences were detected at pH 5, 6, 7 and 9 (Table 2). Among the isolates, DMP_3, KNP_5, KNP_6, KKP_7, KKP_8 and KKP_9 grew at all levels of pH (Figure 8). Six isolates (DMP_3, KNP_5, KNP_6, KKP_7, KKP_8 and KKP_9) grew weakly on pH 5 that indicates acid tolerant and others were found to be sensitive. At pH 6, two isolates showed superior growth, six isolates grew weakly where KNP_4 was sensitive. At pH 7, five isolates were found superior growth and three isolates performed

weak growth where KNP_4 was sensitive. Only three isolates (KNP_6, KKP_8 and KKP_9) showed superior growth but others performed weak growth at pH 8. Superior growth of two isolates (KNP_6 and KKP_9) has been reported on pH 9 which represents alkaline tolerant and others showed weak growth where KNP_4 was sensitive.

Solubilization of insoluble phosphate by isolated PSB

The relative efficiency of the isolates in solubilizing insoluble phosphates like tricalcium phosphate was investigated. It was revealed that the phosphate solubilizing capacity varied between the isolates obtained from different rhizosphere soils. The highest efficient bacterial isolates solubilizing tricalcium phosphate were KNP_4 (21.128 ppm) followed by KKP_7 (19.006 ppm), KNP_5 (17.561 ppm), DMP_3 (16.013 ppm), DMP_2 (14.604 ppm), KNP_6 (11.926 ppm), DMP_1 (11.471 ppm), KKP_8 (4.47 ppm), KKP_9 (4.048 ppm) which became 85.41, 76.02, 69.35, 66.30, 58.423, 49.05, 45.88, 17.88 and 16.19% respectively of the total amount added (25.00 mg P2O5) in 50 mL liquid medium. The results (Table 3) showed that all the bacterial isolates solubilized tricalcium phosphate. Among the bacterial isolates, the highest amount of tricalcium phosphate was solubilized by KNP_4 (21.128 ppm) and the lowest amount of tricalcium phosphate was solubilized by KKP_9 (4.048 ppm) (Figure 9).

Only 0.1% of the total phosphorous from soil is available to plants (Peix *et al.,* 2001; Tilak *et al.,* 2005) and available phosphorous is immediately depleted around the root zone owing to continued plant uptake (Smith *et al.,* 2003). This is important as phosphate is reported as a limiting nutrient (Ezawa *et al.*, 2002) compared to nitrogen for the growth of crops. The rhizosphere bacteria possessing abilities to solubilize phosphate are likely toplay an important role in making the phosphate available to the crops. Phosphate-solubilizing bacterial colonies appear in addition to the other bacterial groups (10% – 15% of the total viable counts). However, in the present study, populations of other bacteria were not quantified. The production of clear zones around the colonies indicates the phosphate-solubilizing ability of the bacterial strain, which is able to enumerate PSB alone. Such cultures have been isolated and the extent of phosphate solubilization was determined quantitatively by biochemical methods. The phosphate-solubilizing ability of the isolate would primarily depend upon the type and concentration of natural substrates, enzymes used temperature, ionic strength and pH of metal ions (McComb *et al.,* 1979). In general, phosphate solubilization seems to be linked with the decrease of the pH of the medium, but this pH decrease is not strictly proportional to the amount of phosphate solubilized. Fankem *et al.* (2006) stated that phosphate solubilization is the result of combined effect of pH decrease and organic acids production. The results (Table 3) also showed that all the bacterial isolates dropped the pH of the medium due to production of acids during solubilization. The highest decrease of pH was observed by KNP_4 (3.14) and lowest by KKP_9 (4.78) from initial pH (5.78).

Table 1. Physiological Characteristics of isolated PSB

Isolate	Shape	Motility	Gram reaction	Catalase	Bromothymol Blue Test	
					Color	Color
DMP1	Oval	Motile	(-) ve	(+) ve	Yellow	Acidic
DMP2	Oval to rod	Motile	(-) ve	(-) ve	Yellow	Acidic
DMP3	Shot rod	Motile	(-) ve	(+) ve	Yellow	Acidic
KNP4	Oval	Motile	(-) ve	(+) ve	Yellow	Acidic
KNP5	Oval	Motile	(-) ve	(+) ve	Yellow	Acidic
KNP6	Shot rod	Motile	(-) ve	(+) ve	Yellow	Acidic
KKP7	Oval	Motile	(-) ve	(+) ve	Yellow	Acidic
KKP8	Shot rod	Motile	(-) ve	(+) ve	Yellow	Acidic
KKP9	Oval	Motile	(-) ve	(+) ve	Yellow	Acidic

Table 2. Tolerance of PSB at different pH levels in Pikovskaya's medium

pH Isolates	5	6	7	8	9
DMP1	-	+	+	+	+
DMP2	-	+	+	+	+
DMP3	+	+	+	+	+
KNP4	-	-	-	+	-
KNP5	+	++	++	+	+
KNP6	+	+	++	++	++
KKP7	+	+	++	+	+
KKP8	+	+	++	++	+
KKP9	+	++	++	++	++

- represent absence of growth, + represent weak growth and ++ = High growth

Table 3. Solubilization of Phosphorus by PSB Isolates

Isolates	Solubilization (ppm)	pH	Net Solubilization (ppm)	% Solubilization
Control	10.64 f	5.78	-	-
DMP1	22.11 d	3.79	11.471	45.88
DMP2	26.65 bc	3.47	14.604	58.42
DMP3	25.24 cd	3.39	16.013	66.30
KNP4	31.77 a	3.14	21.128	85.41
KNP5	28.20 bc	3.34	17.561	69.35
KNP6	22.57 d	3.66	11.926	49.05
KKP7	29.65 ab	3.21	19.006	76.02
KKP8	15.11 e	4.47	4.470	17.88
KKP9	14.69 e	4.78	4.048	16.19
CV (%)	7.93	-	-	-
SE Level of significance	1.28**	-	-	-

Values having same lowercase letters in a column do not differ significantly at 5% level by DMRT

In the study, a linear relationship was observed with the drop in pH of the culture and increase in inorganic phosphate concentration (Figure 10). This has been reported earlier by Chen *et al.* (2006) who stated that the solubilization of tricalcium phosphate in the liquid medium by different strains was accompanied by a significant drop in pH (4.9 to 6.0) from an initial pH of 6.8–7.0 after 72h. In the study, the pH was declined up to 3.14 from 5.78 in 168 h. It is noteworthy that within the culture period of 7 days, pH of the medium was reduced to 3.14 under the given culture condition because of the organic acid production by the bacterial isolates which solubilized organic phosphate. *Bacillus* spp. produces oxalic, 2-ketogluconic acid and succinic acid which are capable to solubilize phosphate (Banik and Dey, 1983). However, Tao *et al.* (2008) reported that there was no correlation between the culture pH and the P mineralization by the Organic Phosphorus Mineralizing Bacteria (OPMB), suggesting that OPMB may have different mechanisms of Phosphorus solubilization. Depending on the composition of the bacterial medium and final pH of the cultured medium, bacterial P solubilization reported in the literature ranges from 31.5 mg/L to 898 mg/L (Chung *et al.* 2005; Chen *et al.* 2006; Son *et al.* 2006; Ma *et al.* 2009; Oliveira *et al.* 2009). The PSB strains exhibit inorganic P-solubilizing abilities ranging between 25–42 μg P/mL (Tao *et al.* 2008). The PSB in conjunction with single super phosphate and rock phosphate reduce the P dose by 25 and 5%, respectively (Sundara *et al.,* 2002). *Pseudomonas putida*, *P. fluorescens* Chao and *P. fluorescens* Tabriz released 51, 29 and 62 % P, respectively.

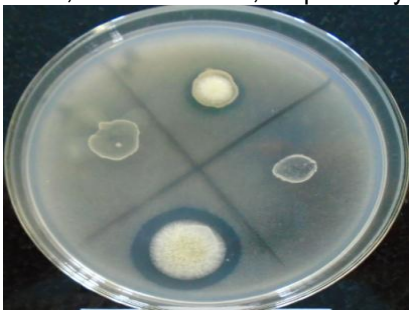


Figure 2. Phosphate Solubilizer forming clear zone

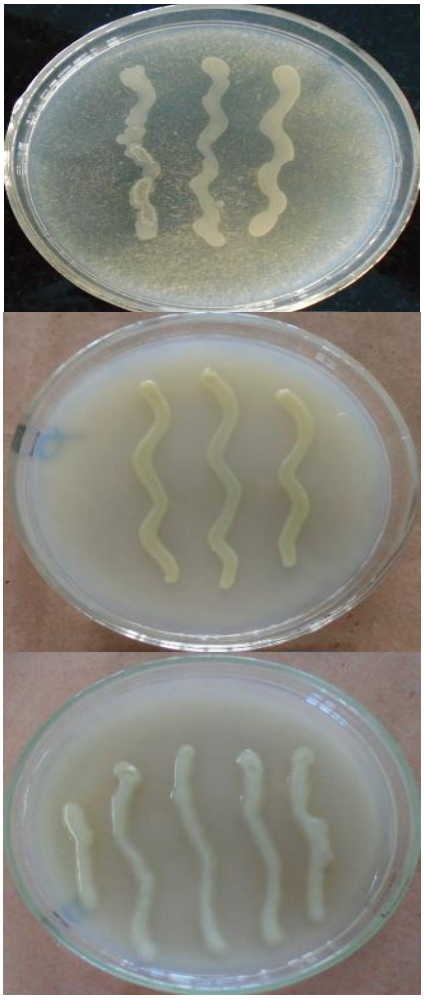
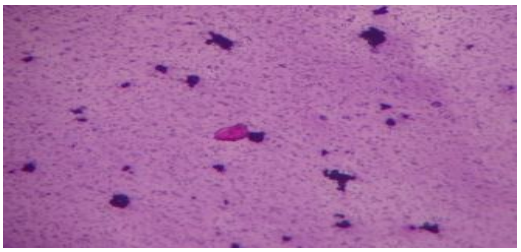


Figure 3. Purification of PSB isolates through streaking method

Figure 4. Mother culture of PSB as slant



[(-) ve = Gram negative]

Figure 5. Microscopic view of Gram staining test of PSB isolates

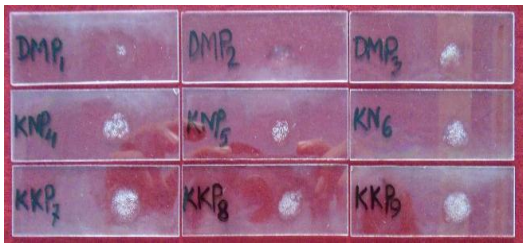


Figure 6. Catalase test of PSB isolates

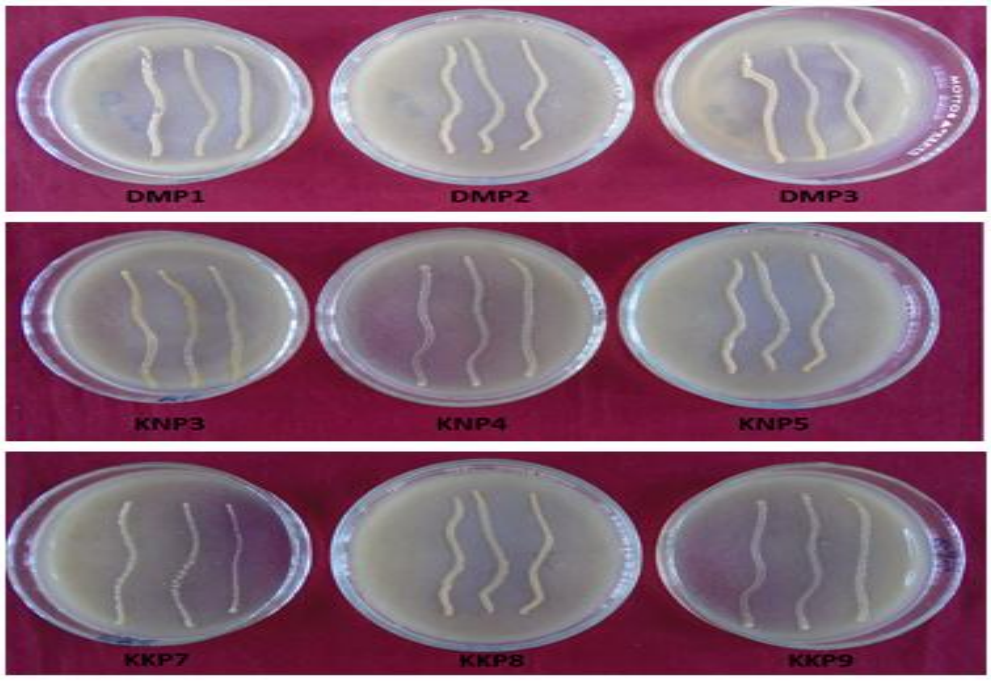
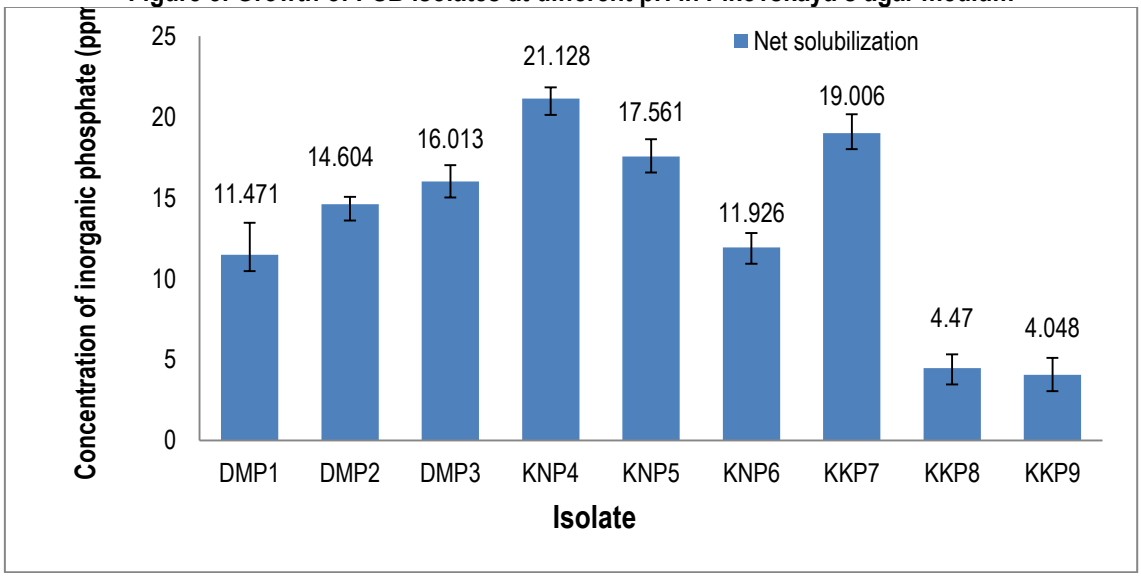


Figure 7. Bromothymol blue test of PSB isolates

Figure 8. Growth of PSB isolates at different pH in Pikovskaya's agar medium



(Vertical bar represent SE value)

Figure 9. Solubilization of phosphorus by PSB isolates

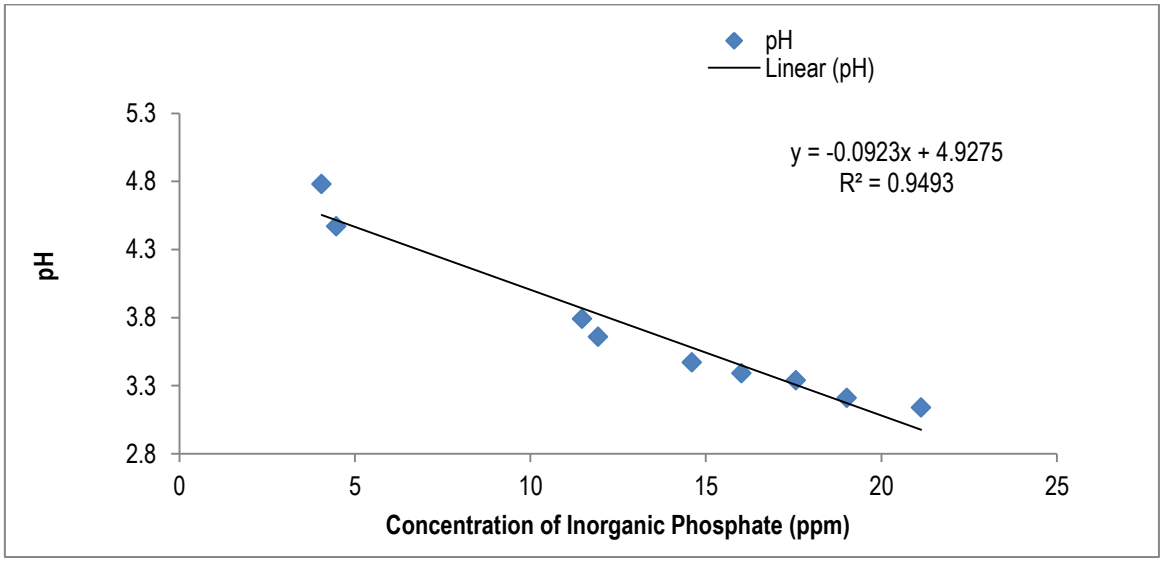


Figure 10. Relationship between Phosphate concentration and pH of Medium

CONCLUSION

In this study the PSB isolates were motile, gram negative, aerobic, anaerobic and acidic in nature. Among the screened nine isolates, KNP_4 was more efficient in terms of phosphorus solubilization. Therefore, this isolate can be used in the production of biofertilizer in order to improve growth of some agricultural crops in P-deficient soils, constituting an interesting alternative to the application of P fertilizers, reducing costs and improving crop yields. Moreover, the present study suggested that more advance research is needed particularly identification and application of potential PSB strain as a phosphate solubilizing inoculant in salt affected soil where conditions are much more complex than those prevailing *in vitro*.

Acknowledgements: Authors are thankful to Dr. Md. Sahidul Islam, Principle Scientific Officer, Bangladesh Agriculture Research Institute (BARI), Gazipur for their keen interest, scholastic guidance, suggestions, supervision, and encouragement and for providing logistic support to accomplish this research.

REFERENCES

Abd-Alla MH. (1994). Phosphatases and the utilization of organic phosphorus by *Rhizobium leguminosarum* biovar *viceae*. Lett. *Appl. Microbiol*. 18: 294-296.

Alok R Mangal RM and Muruhan S. (2013) Isolation and characterization of phosphate-solubi;lizing bacterial species from different crop fields of Salem, Tamil Nadu, India. *Int. J. Nutrition, Pharmacology, Neurological diseases,* 3(1):29-33.

Anonymous. (1997). Fertilizer Recommendation Guide. Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka-1215, Bangladesh, p, 22-29

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, and Young CC. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology*, 34: 33-41.

De Souza BDMJ, Nair S and Chandramohan D. (2000). Phosphate solubilizing bacteria around Indian peninsula. *Indian Journal of Marine Sciences*, 29: 48-51.

deFreitas JR, Banerjee MR and Germida JJ. (1997). Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of Canola (*Brassica napus* L.). *Biol. Fertil. Soils,* 24: 358-364.

Ezawa T. Smith SE and Smith FA. (2002). P metabolism and transport in AM fungi. *Plant and Soil*, 244: 221-230.

Goldstein AH. (1986). Bacterial phosphate solubilization. Historical perspective and future prospects. *Am. J. Alt. Agric*. 1: 57-65.

Grover R. (2003). Rock phosphate and phosphate solubilizing microbes as a source of nutrients for crops. Dissertation,

Department of biotechnology and environmental sciences, Thapar Institute of engineering and technology, Patiala, India.

Gyaneshwar P, Kumar G, Parekh L and Poole P. (2002). Role of microorganims in improving P nurtient of plants. *Plant Soi,* 245:83-93.

Lindsay WLP, Vlek LG and Chien SH. (1989). Phosphate minerals. *In:* Minerals in soil environment. 2nd ed. (Eds. J.B. Dixon and S.B. Weed). Soil Science Society of America, Madison, USA, p, 1089-1130.

McComb RB, Bowers GN and Posen S. (1979). Alkaline phosphatases. Plenum Press, New York and London, p, 986.

Mehta S. and Nautiyal C S. (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Curr. Microbiol*. 43: 51-56

Mondal D. Islam MS, Hoque MF, Rafiq M and Ahsan SM. (2016). Screening and Isolation of Salt Tolerant Bacteria from Tidal Floodplain Soils of Bangladesh. *Octa Journal of Biosciences*, 4(1):11-16.

Norrish K and Rosser H. (1983). Mineral phosphate, *In:* Soils, an Australian viewpoint. Academic press, Melbourne, CSIRO/London, UK, Australia, p, 335-361.

Olsen SR Cole CV Watanable FS and Dean EA. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Dept. Agril. Circ, p, 929.

Peix A, Rivas-Boyero AA, Mateos PF, Rodriguez-Barrueco C, Martínez-Molina E and Velazquez E. (2001). Growth promotion of chickpea and barley by a phosphatesolubilizing strain of Mesorhizobium mediterraneum under growth chamber conditions. *Soil Biology and Biochemistry*, 33: 103-110.

Richardson AE. (2001). Prospects for using soil microorganism to improve the acquisition of phosphorus by plants. *Aust. J. Plant Physiol*, 28: 897-906.

Rodrı´guez H and Fraga R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv*, 17: 319-339.

Seshadri S, Ignacimuthu S, and Lakshminarsimhan C. (2002). Variations in heterotrophic and phosphate solubilising bacteria from Chennai, southeast coast of India. *Indian Journal of Marine Sciences*, 31: 69-72.

Smith SE, Smith FA and Jakobsen I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology*, 133:16-20.

Stevenson FJ. (1986). Cycles of soil carbon, nitrogen, phosphorus, sulphur micronutrients, Wiley, New York.

Tao GC, Tian SJ, Cai MY and Xie GH. (2008). Phosphate- solubilizing and mineralizing abilities of bacteria isolated from soils. *Pedosphere*, 18 (4): 515-523.

Thakuria D, Talukdar NC, Goswami C, Hazarika S, Boro RC and Khan MR. (2004). Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. Curr. Sci. 86: 978-985.

Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Shekhar Nautiyal C, Mittal S, Tripathi AK and Johri BN. (2005). Diversity of plant growth and soil health supporting bacteria. *Current Science*, 89: 136-150.

Vassileva VM, Vassilev N, Fenice M, Federici F. (2001). Immobilized cell technology applied in solubilization of insoluble inorganic (rock) phosphate and P plant acquisition. *Bioresource Technol.* 79: 263-271.

Vessey JK. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil,* 255: 571-586.

Widawati S. (2011). Diversity and phosphate solubilization by bacteria isolated from laki island coastal ecosystem. *Biodiversitas*. 12(01): 17-21.

Yadav KS and Dadarwal KR. (1997). Phosphate solubilization and mobilization through soil microorganisms. *In:* Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production (Dadarwal R. K.,ed). Scientific Publishers, Jodhpur, India, p, 293-308.

Yahya A and Al-Azawi SK. (1989). Occurrence of phosphate-solubilizing bacteria in some Iraqi soils. *Plant Soil,* 117:135-141.

Source of Financial Support: None.
Conflict of interest: None. Declared.