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# Combined use of phosphate solubilizing bacteria and poultry manure to enhance the growth and yield of mung bean in calcareous soil

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## Abstract

*Although most of the soils are rich in total phosphorus (P), but its large portion remains unavailable to plants and is considered as a limiting factor for plant growth especially in alkaline calcareous soils. Phosphate solubilizing bacteria (PSB) is a group of bacteria that enhance the P availability by solubilizing hydrolysable inorganic P compounds in addition to mineralization of soil organic P. Sixteen bacterial isolates were isolated from the rhizosphere of mung bean and tested in lab for phosphate solubilization and phosphatase activities. Five bacteria were found positive for P solubilization as well as in vitro production of phosphatase enzyme. A pot experiment was conducted to evaluate the selected five phosphate solubilizing bacteria in combination with poultry manure (PM) on growth and yield of mung bean. Results showed that inoculation with PSB improved the growth and yield of mung bean in presence as well as absence of PM as compared with un-inoculated control. However, PSB isolates were most effective for enhancing yield and growth of mung bean in the presence of PM compared with their use in absence of PM, most probably due to their phosphatase activity. Maximum increases in pods number per plant (80%), 100 grain weight (57%), and grain yield per plant (36%) were observed by combined use of isolate S5 and PM as compared to un-inoculated control without PM. Combined application of PSB and PM also caused significant increase in phosphatase activity of the rhizosphere (79% over control) and available P contents (45% over control) in soil. The most efficient bacterial isolate (S5) was identified as Bacillus thuringiensis on the basis of rrs (16S rRNA) gene sequencing. It is concluded that multi-trait PSB with phosphatase activity in combination with organic amendments could be more convincing to enhance P bioavailability for plants especially in alkaline calcareous soils.*

**Keywords:** phosphatase; organic amendments; phosphorus availability; PSB; *Bacillus*

## Introduction

Phosphorus is a vital nutrient and is a key element for plant growth and development that makes up about 0.2% of the dry weight of plant (Gyaneshwar *et al*., 2002; Dorahy *et al*., 2004). It is vital for normal functions of plants due to its involvement in metabolic processes during energy transformation (Achal *et al*., 2007). Phosphorus nutrition plays crucial role in root development, seed and flower formation, strengthening of stem and stalk, fixation of nitrogen in legumes, and diseases resistance of almost all plant species (Gyaneshwar *et al*., 2002; Hao *et al*., 2002). Phosphorus deficiency is one of the major factors which affect productivity and growth of crops (Raghothama and Karthikeyan, 2005) and it also results in formation of weak stem and small leaves of plants (Ranjan *et al*., 2013). Plants available P is deficient in about 80 to 90% soils of the arid and semiarid areas of the world (Mehrvarz and Chaichi, 2008). Most soils contain high content of total phosphorus (Abou El-Yazeid and Abou-Aly, 2011) but the availability of P to plants may be limited depending upon the chemical nature of the soils and nutritional status of soil (Hussain *et al*., 2013a). Plants absorb P as anions of phosphate ($HPO_4^{--}$ or $H_2PO_4^-$) from the soil solution but, these anions of phosphate are also very reactive and become unavailable to plants due to fixation or precipitation with aluminium and iron in acidic soils (Hao *et al*., 2002; Dorahy *et al*., 2004) and with calcium and magnesium in alkaline soils (Mahantesh and Patil, 2011). A large part of P applied through different chemical fertilizers becomes unavailable to plants due to fixation or precipitation with soil minerals (Khan *et al*., 2007; Mittal *et al*., 2008). Management of P fertilization through chemical methods usually affects the cost of production and also soil health (Jha *et al*., 2011). Whereas, key objective of management of soil P should be to get optimum yield of crop and reduce the fixation of phosphorus in the soil (Fasim *et al*., 2002).

In agriculture, phosphate solubilizing bacteria (PSB) are well documented for their ability to improve P availability to crop plants (Kannaiyan *et al.*, 2004; Bucher, 2007). The capacity of P solubilization by microbes is considered an important mechanism for providing P nutrients to plants. Solubilization of bound P by PSB is carried out by the production of low molecular weight (LMW) organic acids, by which their functional groups

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such as carboxyl and hydroxyl are bonded to the phosphate thus making them available for plants (Vessey, 2003). Additionally, PSB can also produce phosphatase enzymes that efficiently hydrolyze organic forms of phosphate compounds (Chen *et al*., 2006; Sharma *et al*., 2011). Additionally, PSB can also increase the availability of P for plants by other indirect ways like productions of phytohormones, ACC deaminase activity and siderophores production ability (Hussain *et al*., 2013a). Soil or seed inoculation with phosphate solubilizing microorganisms is well-known to increase solubilization of soil P resulting in increased yield of crop (Hussain *et al*., 2013b).

Organic amendments can also be used for improving availability of soil P (Wolkowski, 2003) to crop plants. Higher crop yield has been reported in fields where manures were applied compared to inorganic sources at same level of P (Nikus *et al*., 2004). The organically bound P in soils is mainly present in the form of nucleotides, inositol phosphate and phospholipids (Turner *et al*., 2002). Plants do not have the ability to uptake and utilize soil organic P (Pradhan and Sukla, 2005) so it must be mineralized into inorganic phosphate before uptake by plants (Chen *et al*., 2006). Among organic amendments, poultry manure (PM) is a concentrated source of P and its application improves plant growth by producing higher values for leaf area index, biomass, and height (Boateng *et al*., 2006). Soil microorganisms act on added organic amendments, decompose organic molecules and release inorganic P by phosphatase enzymes thus improve the phosphorus availability in the soil for plant growth (Browne *et al*., 2009). Keeping in view the role of PSB for enhancing P availability by phosphate solubilization and mineralization of soil organic P, this study was conducted to examine the effectiveness of PSB having phosphatase activity in combination with PM as organic amendment to improve P availability for better yield and growth of mung bean.

## Material and Methods

### Isolation and characterization of phosphate solubilizing bacteria (PSB) with phosphatase activity

Sixteen rhizobacteria from rhizosphere of mung bean plant were isolated. Plants were uprooted and taken to the lab in polythene ziplock bags and bulk soil around the roots was detached by mildly shaking the plant. The rhizospheric soil was collected in sterilized water by dipping and shaking of roots under sterilized conditions. Dilution plating technique was used for the isolation of rhizobacteria. Repeated streaking method was followed for purification of rhizobacteria using glucose peptone agar media. These rhizobacterial isolates were verified for their phosphate solubilizing potential by using the Pikovskaya's agar medium (Pikovskaya, 1948) provided with calcium phosphate as substrate. The qualitative phosphate solubilization potential was estimated by observing the clear halo zones around colonies on Pikovskaya's agar medium. Further, phosphate solubilizing index of these five PSB was calculated by following formula (Edi-Premono *et al*., 1996).

Phosphate Solubilizing Index (PSI) =

(Colony diameter + Clearing zone)/(Colony diameter)

Phosphate solubilization potential of these isolates was confirmed in Pikovskaya's medium in liquid form with calcium phosphate at a conc. of 5 g $L^{-1}$ as described by Chung *et al*. (2005). Furthermore, acidic and alkaline phosphatase activity of PSB was estimated by following the method used by Eivazi and Tabatabai (1977). These phosphate solubilizing rhizobacterial isolates with phosphatase activity were evaluated for their auxins production, as equivalents of indole acetic acid (IAA) both in the presence and absence of L-tryptophan (Sarwar *et al*., 1992). Auxin compounds were analyzed by spectrophotometer, using Salkowski coloring reagent. Moreover, these rhizobacterial strains were also tested for siderophores production (Schwyn and Neilands, 1987), oxidase activity (Kovaks, 1956), catalase activity (Graham and Parker, 1964), Gram staining (Holt *et al*., 1994) and cyanide production (Castric, 1975).

### Pot experiment

An experiment was carried out in pots in the net house under natural conditions to evaluate the efficiency of phosphate solubilizing bacteria (PSB) having phosphatase activity alone and in combination with poultry manure (PM) for improving growth and yield of mung bean [*Vigna radiata* (L.) Wilczek]. For this, pots were filled with sandy clay loam soil having pHs 8.3, calcium carbonate ($CaCO_3$) 24.6 g $kg^{-1}$, bicarbonates ($HCO_3^{--}$) 4.2 mmolc $L^{-1}$, saturation percentage 33%, ECe 1.31 dS $m^{-1}$, CEC 5.23 $Cmol_c$ $kg^{-1}$, organic matter 0.58%, total nitrogen 0.07%, extractable potassium 112 mg $kg^{-1}$ and available P 7.54 mg $kg^{-1}$. Soil was homogenized, air dried under room temperature, ground and sieved through 2.5 mm mesh and 10 kg of soil was filled in each pot. Prior to pot filling, soil was thoroughly mixed with poultry manure (PM) @ of 4 percent, on dry weight basis. Poultry manure with 8.4 pH, 4.2% total N, 2.3% total P, 1.7% total K and 18% organic matter was used in this experiment. Five phosphate solubilizing bacteria (PSB) having phosphatase activity were used for inoculation of mung bean seeds. Selected isolates were used for preparation of inoculum in glucose

peptone broth medium. The loopfull of selected PSB isolates were inoculated into flasks containing glucose peptone broth and inoculated flasks were placed in an incubator at 28±1°C for 3 days. Centrifugation was used for collecting bacterial cells at 4500 rev $min^{-1}$ for 20 minutes. Then cells were washed and suspended in sterilized phosphate buffer saline (PBS) and uniform cell density ($10^7$-$10^8$ CFU $mL^{-1}$) was achieved by retaining optical density (OD=0.45) at 535 nm. Before coating the seeds, sterile peat was injected with inoculum of selected PSB isolates and incubated for 24 hrs under optimum temperature (28±1°C). Seed inoculation was done by using peat and sugar solution (10%) as sticky material and clay as carrier material. For the control (un-inoculated) treatment, autoclaved inoculum suspension was used for coating the seeds.

Five mung bean seeds inoculated with 5 different bacteria were sown in each pot. To separate the effect of PSB isolates and PM, mung bean seeds coated with autoclaved inoculum suspension (un-inoculated) were also sown in pots containing soil amended with and without PM. Three plants were maintained by manual thinning after 7 days of germination. Pots were organized in factorial fashion under completely randomized design (CRD) set up, in net house under optimum temperature and ambient light. Three replications were used. Recommended dose of NPK fertilizers (@ 25-60-60 Kg $ha^{-1}$) was applied as $CO(NH_2)_2$ (urea), single super phosphate (SSP), and sulphate of potash ($K_2SO_4$), respectively, along with other recommended agronomic practices. Nitrogen was applied in two splits, first dose of N at sowing time while second dose after 20 days of sowing was applied. Full doses of K and P fertilizers were applied at sowing time as basal dose for all treatments. Tap water was used for irrigation. At maturity, crop was harvested and data regarding growth and yield attributes were taken by following standard procedures. The rhizosphere soil was collected after harvesting for analysis of available P contents and alkaline phosphatase activity (Eivazi and Tabatabai, 1977).

## Sequencing of *rrs (16S rRNA)* gene of PSB isolate for identification

For extraction of genomic DNA of the most efficient PSB isolate, CTAB/Chloroform isoamyl alcohol method was used (Wilson, 1987). Target *rrs* (*16S rRNA*) gene was amplified through PCR by the use of universal primers. Amplification, purification and sequencing process of polymerase chain reaction was carried out by Macrogen Inc., Korea. The sequence was paralleled with known nucleotide sequences by BLastN tool of NCBI (http://www.ncbi.nlm.nih.gov/BLAST). The sequence of PSB isolate S5 was submitted to the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) with accession number KT750960.

## Statistical Analysis

Computer based statistical software, Statistix-8.1® (Analytical Software, Tallahassee, USA) was used for analyzing data statistically and for comparing means, Duncan's Multiple Range Test (DMRT) was used.

## Results

In the present study, total sixteen rhizobacteria were isolated from rhizosphere of mung bean and only five rhizobacterial isolates showed phosphate solubilization ability as well as phosphatase activity. The phosphate solubilizing index (PSI) of five rhizobacterial isolates ranged from 2.33 to 3.17 and maximum PSI was observed by S5. Quantitative phosphate solubilizing assay also showed that these isolates had ability to solubilize inorganic P and isolate S5 caused maximum solubilization of inorganic P (Table 1). These five PSB isolates also had ability to produce phosphatase enzymes (acid and alkaline). The acid phosphatase activity ranged from 7.92-19.54 µg PNP $g^{-1}$ $h^{-1}$ whereas alkaline phosphatase activity ranged from 5.97-13.57 µg PNP $g^{-1}$ $h^{-1}$ by all five PSB isolates. Isolate S5 showed maximum acid and alkaline phosphatase activity compared to other PSB isolates (Table 1). All the PSB isolates produced auxins as indole acetic acid (IAA)

**Table 1: Phosphate solubilizing index and phosphatase activity of PSB isolates**

| Isolates Code | Phosphatase Solubilizing Index (PSI) | Available phosphorus (µg $mL^{-1}$) | Phosphatase Activity (µg PNP $g^{-1}$ $h^{-1}$) | | IAA equivalents (µg $mL^{-1}$) | |
|---|---|---|---|---|---|---|
| | | | Acidic | Alkaline | Without L-TRP | With L-TRP |
| **S1** | 2.75±0.31 | 417.70±21.17 | 12.95±1.02 | 09.60±1.20 | 0.74±0.3 | 10.91±0.5 |
| **S2** | 2.64±0.07 | 399.58±10.84 | 08.31±0.60 | 05.79±1.30 | 1.69±0.3 | 8.11±1.4 |
| **S3** | 2.85±0.23 | 529.38±11.91 | 08.65±0.60 | 09.11±0.50 | 0.33±0.2 | 12.43±0.4 |
| **S4** | 2.33±0.05 | 365.92±10.03 | 07.92±0.60 | 06.94±1.10 | ND | 6.29±0.3 |
| **S5** | 3.17±0.30 | 379.16±14.98 | 19.54±0.63 | 13.97±0.84 | 1.01±0.3 | 16.13±0.6 |

ND =not detectable; (Average of three replicates ± SE)

equivalents both in presence and absence of L-tryptophan except only one isolate (S4) which did not produce IAA equivalent in absence of L-tryptophan. Isolate S2 produced maximum IAA equivalents (1.69 µg $mL^{-1}$) in absence of L-tryptophan while in presence of L-tryptophan maximum IAA equivalents (16.13 µg $mL^{-1}$) were observed with PSB isolate S5. From five PSB isolates, 3 isolates (S1, S3 and S4) were Gram negative and 2 isolates (S2 and S5) were Gram positive (Table 2). All five PSB isolates found positive for the production of catalase enzyme as they produced bubbles by adding 2-3 drops of $H_2O_2$ on cells of bacteria placed on glass slide. While in case of oxidase activity, 2 isolates (S2 and S4) produced cytochrome oxidase enzyme as they showed purple color and remaining 3 isolates (S1, S3 and S5) failed to produce purple color. Data showed that 3 isolates (S2, S3, and S4) exhibited the cyanide production activity while the isolates (S1 and S5) did not show cyanide production activity.

**Table 2: Characterization of selected bacterial isolates**

| Isolate Code | Gram staining | Oxidase test | Catalase test | HCN |
|---|---|---|---|---|
| S1 | - | - | + | - |
| S2 | + | + | + | + |
| S3 | - | - | + | + |
| S4 | - | + | + | + |
| S5 | + | - | + | - |

## Pot trial

The most efficient PSB isolates containing phosphatase activity were evaluated alone as well as in combination with PM to enhance P availability to mung bean for improving its growth and yield in a pot experiment. It was perceived from the data that sole inoculation of all PSB isolates boosted the yield and growth attributes of mung bean. However, effectiveness of PSB isolates was further improved when applied in combination with PM and response of all isolates was variable for improvement in growth and yield attributes of mung bean, either used alone and/or in combination with PM.

Data presented in Table 3 indicated that statistically substantial increase in plant height and fresh weight was detected by inoculation with all PSB isolates alone and in combination with PM compared to un-inoculated control. All PSB isolates caused increase in root length but results were statistically at par by inoculation with S2 and S4 in absence of PM with un-inoculated control. However, increase in root length was statistically significant when all PSB isolates were used with PM as compared to un-inoculated control. Plant dry weight was significantly increased by inoculation of S3 and S5 without PM, although, increase in plant dry weight was also statistically significant by inoculation of all PSB isolates in combination with PM as compared to un-inoculated control. Combined use of PSB isolate S5 and PM caused maximum increases in plant height (62%), root length (65%), plant fresh weight (74%) and plant dry weight (67%) as compared to un-inoculated control without PM, which was up to 51, 47, 63 and 57% more, respectively, as compared to un-inoculated control with PM.

Data (Table 4) showed that inoculation of PSB isolates with and without PM caused significant increase in pods number per plant and yield of grains per plant as compared to un-inoculated control. Only inoculation of isolate S1 without PM caused non-significant increase in 100-grain weight, while all other PSB isolates significantly improved 100-grain weight as compared to un-inoculated control either used with and/or without PM. Maximum increases in pods number per plant (80%), 100 grain weight (57%), and grain yield per plant (36%) was observed by combined use of PSB isolate S5 and PM as compared to un-inoculated control without PM, which was up to 74, 44 and 32 % more, respectively, as compared to un-inoculated control with PM.

Inoculation of PSB isolates alone as well as in combination with PM significantly enhanced the nitrogen (N), phosphorus (P) and potassium (K) contents in straw as compared to un-inoculated control (Table 5). Inoculation of PSB isolates without PM caused 18 to 46%, 9 to 46% and 4 to 44% increases in N, P and K contents of straw,

**Table 3: Effect of PSB inoculation and poultry manure (PM) on growth parameters of mung bean**

| Treatment | Shoot length | | Root length | | Plant fresh weight | | Plant dry weight | |
|---|---|---|---|---|---|---|---|---|
| | Without PM | With PM | Without PM | With PM | Without PM | With PM | Without PM | With PM |
| Control | 14.5h | 15.6gh | 8.07f | 9.0ef | 10.9h | 11.6gh | 2.6f | 2.8ef |
| S1 | 17.6ef | 21.3bc | 10.4c-e | 12.5ab | 15.6e | 17.7bc | 3.2b-f | 3.8a-c |
| S2 | 16.6fg | 19.9cd | 9.3d-f | 10.4cd | 12.3fg | 17.2cd | 3.0d-f | 3.7a-d |
| S3 | 17.6ef | 22.2ab | 10.0c-e | 12.1ab | 15.2e | 18.7ab | 3.5a-e | 3.9ab |
| S4 | 16.3fg | 19.1d-e | 9.4d-f | 11.2bc | 13.4f | 17.9a-c | 3.1c-f | 3.8a-c |
| S5 | 18.9de | 23.5a | 11.3bc | 13.3a | 16.1de | 19a | 3.7a-d | 4.0a |
| *LSD Value* | *1.5345* | | *1.3790* | | *1.2004* | | *0.7099* | |

Mean(s) sharing the same letter(s) do not differ significantly at $p \leq 0.005$

respectively, over un-inoculated control. However, when these PSB isolates were applied with PM, increases in N, P and K contents were from 56 to 68 %, 55 to 91% and 53 to 92%, respectively, over their respective un-inoculated control. Most efficient PSB isolate was S5 for improving N, P and K contents in straw and caused maximum increase in absence as well as presence of PM. Similarly, N, P and K contents of grains were also influenced by the use of PSB isolates and PM as compared to control (Table 6). Maximum increases in N, P and K contents of grain were observed with combined use of PSB isolate S5 and PM, which were about 54, 50 and 82%, respectively, more as compared to un-inoculated control but amended with PM.

Rhizosphere soil analysis showed (Table 7) that PSB isolates alone as well as in combination with PM caused increase in soil phosphatase activity (alkaline). However, increase in soil phosphatase activity was more prominent by PSB isolates in presence of PM. Maximum soil phosphatase activity was observed by combined use of isolate S5 in presence of PM which was statistically significant from all other treatments. Moreover, PSB isolates also enhanced available P contents in the rhizosphere soil and effectiveness of PSB isolates was further improved for increasing soil available P contents, when used in combination with PM. It was observed that PSB isolate S5 caused statistically significant and maximum increases in soil available P contents alone (22% over respective un-inoculated control) as well as in combination with PM (64.7% over respective un-inoculated control). According to linear regression analysis (Figure 1), rhizosphere soil phosphatase activity (alkaline) was significantly associated with available P contents of soil ($r$ = 0.82), P contents in straw ($r$ = 0.90), P contents in grains ($r$ = 0.82) and grain yield of mung bean ($r$ = 0.91).

**Table 4: Effect of PSB inoculation and poultry manure (PM) on yield and yield components of mungbean**

| Treatment | No. of pods plant$^{-1}$ | | 100-grain weight | | Grain yield plant$^{-1}$ | |
|---|---|---|---|---|---|---|
| | Without PM | With PM | Without PM | With PM | Without PM | With PM |
| **Control** | 11.3h | 11.6h | 2.80f | 3.1ef | 3.3h | 3.4h |
| **S1** | 16.0e | 18.3bc | 3.40d-f | 4.1ab | 3.7f | 4.1cd |
| **S2** | 14.6fg | 17.6cd | 3.60b-e | 3.8b-d | 3.5g | 4.3b |
| **S3** | 15.6ef | 19.3ab | 3.70b-e | 4.0ac | 3.6f | 4.2bc |
| **S4** | 13.6g | 17.3cd | 3.50c-e | 3.8b-d | 3.6fg | 4.0de |
| **S5** | 16.6de | 20.3a | 3.90a-d | 4.5a | 3.9e | 4.4a |
| ***LSD Value*** | *1.1760* | | *0.5936* | | *0.1162* | |

**Table 5: Effect of PSB inoculation and poultry manure (PM) on nutrient concentrations in straw of mung bean**

| Treatment | Nitrogen (%) | | Phosphorus (%) | | Potassium (%) | |
|---|---|---|---|---|---|---|
| | Without PM | With PM | Without PM | With PM | Without PM | With PM |
| **Control** | 1.10 l | 1.20 k | 0.11i | 0.14h | 1.30i | 1.40hi |
| **S1** | 1.30 j | 1.71e | 0.13f | 0.19c | 1.62fg | 2.18ab |
| **S2** | 1.52 i | 1.76 b | 0.13g | 0.18c | 1.50gh | 2.10bc |
| **S3** | 1.57 g | 1.72 d | 0.15d | 0.20b | 1.75ef | 2.55a |
| **S4** | 1.60 h | 1.81 c | 0.12h | 0.17d | 1.48gh | 1.99cd |
| **S5** | 1.61f | 1.85 a | 0.16e | 0.21a | 1.87de | 2.50a |
| ***LSD Value*** | *0.0491* | | *0.0126* | | *0.1689* | |

**Table 6: Effect of PSB inoculation and poultry manure (PM) on nutrient concentrations in grains of mung bean**

| Treatment | Nitrogen (%) | | Phosphorus (%) | | Potassium (%) | |
|---|---|---|---|---|---|---|
| | Without PM | With PM | Without PM | With PM | Without PM | With PM |
| **Control** | 1.20k | 1.3j | 0.18g | 0.19cd | 0.95h | 1.35cd |
| **S1** | 1.41i | 2.2c | 0.20fg | 0.26ab | 1.12g | 1.46b |
| **S2** | 1.67g | 2.11d | 0.22e-g | 0.26ab | 1.22ef | 1.46b |
| **S3** | 1.70g | 2.31b | 0.22c-e | 0.27a | 1.16fg | 1.68a |
| **S4** | 1.53h | 1.92e | 0.21c-e | 0.24bc | 1.28de | 1.38bc |
| **S5** | 1.81f | 2.45a | 0.23d-f | 0.28a | 1.14fg | 1.75a |
| ***LSD Value*** | *0.0543* | | *0.0253* | | *0.0964* | |

Mean(s) sharing the same letter(s) do not differ significantly at $p \leq 0.005$

**Table 7: Effect of PSB inoculation and poultry manure (PM) rhizosphere phosphatase activity**

| Treatment | Rhizosphere Soil Phosphatase Activity (µg PNP $g^{-1}$dwt soil-$h^{-1}$) | | Rhizosphere Soil Available P ( mg $kg^{-1}$) | |
|---|---|---|---|---|
| | Without PM | With PM | Without PM | With PM |
| Control | 167.57 i | 203.82 fg | 8.56 e | 10.65 cd |
| S1 | 212.79 fg | 283.25 d | 10.15 c-e | 13.21 ab |
| S2 | 202.03 fg | 306.54 c | 9.97 c-e | 12.71 ab |
| S3 | 213.19 fg | 327.74 b | 10.29 c-e | 13.85 a |
| S4 | 186.61 h | 246.88 e | 9.07 de | 11.66 bc |
| S5 | 216.48 f | 344.59 a | 10.47 c-e | 14.10 a |
| LSD | 13.833 | | 1.9248 | |

Mean(s) sharing the same letter(s) do not differ significantly at $p \leq 0.005$

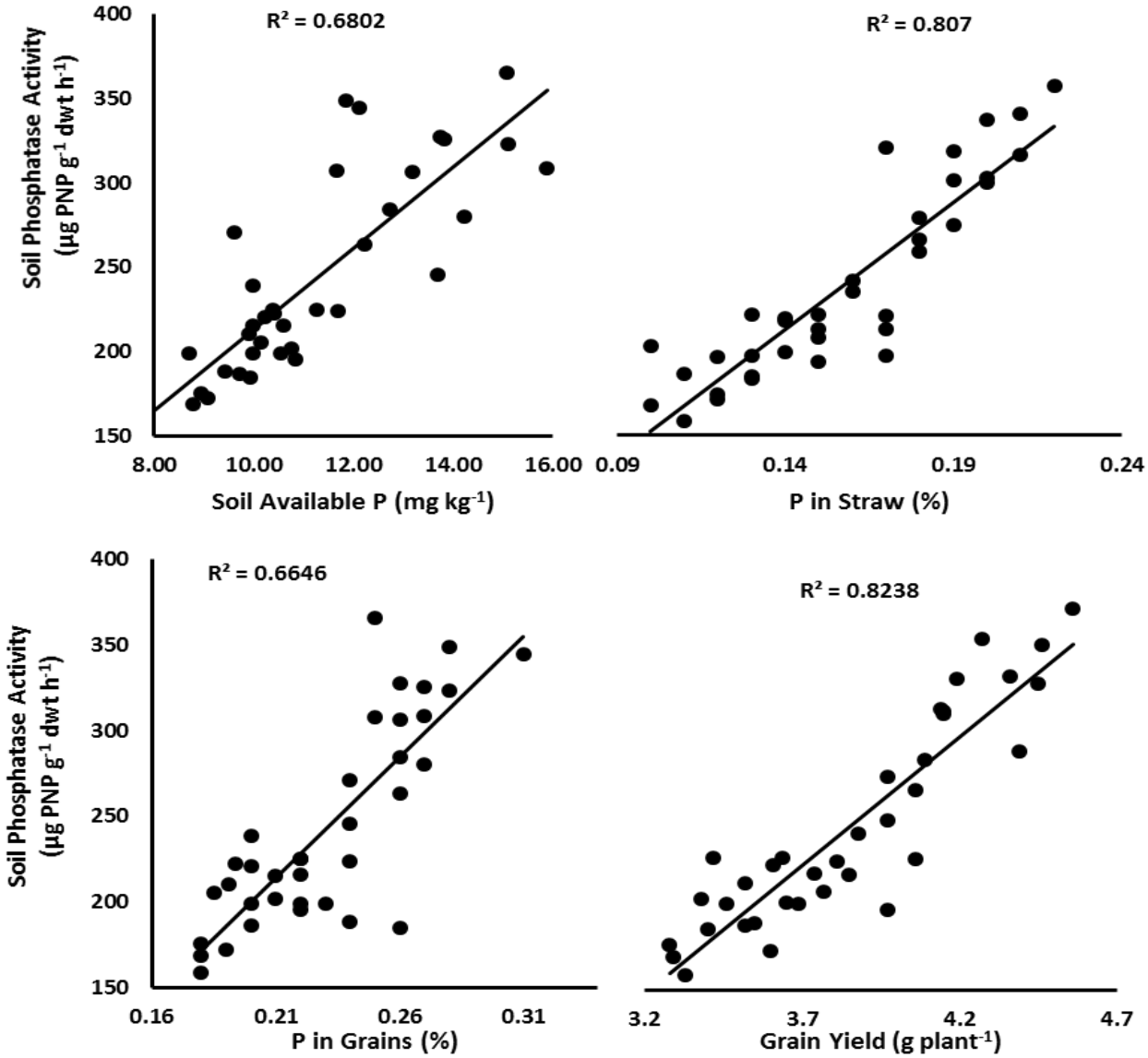


**Figure 1: Correlation between rhizosphere phosphatase activity with soil available P, P in straw, P in grains and Grain yield**

The most efficient PSB isolate S5 was identified and named as *Bacillus thuringiensis* strain S5 on the basis of *rrs* (*16S rRNA*) gene sequencing. The BlastN analysis of the obtained sequence showed that PSB isolate S5 had 99% similarity genus *Bacillus thuringiensis*. Closest match of selected PSB strain was *Bacillus thuringiensis* (NC_005957). The Baispair length of sequence was 986 and its tentative phylogenetic group was *Bacillaceae.*

## Discussion

Multi-trait bacteria with phosphate solubilizing capability and phosphatase activity would have an advantage for increased availability of phosphorus to crop plants when used in combination with organic amendments by mobilization of soil P through solubilization of fixed soil phosphorus and mineralization of organic P sources. In present investigation, rhizobacteria were isolated from mung bean rhizosphere and assessed for phosphate solubilization as well as phosphatase activity. Further, PSB with phosphatase activity were evaluated to increase growth and yield of mung bean alone as well as in combination with PM as an organic amendment. Herein, PSB isolates with phosphatase activity improved the plant growth and enhanced the root length, plant height and fresh and dry biomass of mung bean. It was observed that, effectiveness of PSB isolates was greatly improved for enhancing plant growth and yield when used in combination with PM. The improvement in plant growth and yield might be contributed by their ability to produce auxins. Selected PSB isolates produced auxins when L-tryptophan was present and only one PSB isolate did not produce auxins in absence of L-tryptophan. Therefore, most probably production of auxins by PSB isolates might cause improvement in root system (Khalid *et al*., 2004) of mung bean which ultimately resulted in better plant growth and yield. Talboys *et al*. (2014) reported that auxins biosynthesis by *Bacillus amyloliquefaciens* FZB42 not only improves root production but also increases the root exudation of organic C as well as re-modulates expression Pi transporter. Although, production of phytohormones is considered as one the most common mechanisms of PGPR for plant growth promotion and auxins might play important role in plant growth promotion (Naveed *et al*., 2014).

However, this improvement in yield as well as in growth of mung bean might also be endorsed to some other plant growth promoting activities of these PSB isolates which could not be ruled out. Principally, herein, it is inferred that improvement in plant growth and yield of mung bean might be attributable to activity of these PSB isolates to solubilize fixed P and biosynthesis of phosphatase enzymes. Use of PSB isolates caused significant increase in rhizosphere phosphatase activity (alkaline) of mung bean rhizosphere. A significant linear link between rhizosphere activity of phosphatase and rhizosphere available phosphorus contents of mung bean was existed. Moreover, increase in phosphatase activity and available P contents in rhizosphere soil of mung bean were further improved when these PSB isolates were used in combination with PM. It is much likely that the capability of these PSB isolates to produce phosphatase enzymes and solubilize fixed phosphorus might cause increase in phosphatase activity and available P contents of rhizosphere soil. Phosphatase enzymes are able to carry out mineralization of organic P sources (Tazisong *et al.*, 2015) and it might be the reason that available P contents were increased in rhizosphere soil in presence of PM. Phosphorus mobilizing bacteria solubilize inorganic P by efflux of proton and organic ions and mineralize the organic P by release of phosphatase and cellulolytic enzymes and/or through ligand exchange reactions (Ryan *et al*., 2001). In presence of PM, prominent increase in phosphatase activity and available P contents in rhizosphere of mung bean might also be attributable to improved organic matter status of soil. Poultry manure is a source of organic carbon which might improve soil organic matter status of soil and enhance the microbial activities (Zhen *et al.*, 2014; Lu *et al*., 2015) and their abilities to produce extra-cellular enzymes (Tazisong *et al*., 2015). Therefore, it might be the case that the efficiency of PSB isolates was improved in presence of PM, and caused a better yield growth of mung bean crop. Phosphate solubilizing bacteria not only caused enhancement in available P contents in rhizosphere soil but P contents in straw and grains of mung bean were also greatly increased both in presence as well as absence of PM. Phosphorus is a key nutrient for plant growth and its deficiency reduces the productivity of agricultural crops (Hunter *et al*., 2014). It takes part in plant's physiological features like enzymatic signaling, energy transfer, glycolysis, membrane functionality and photosynthesis (White and Hammond, 2008; Tazisong *et al*., 2015). Therefore, better P nutrition of mung bean might improve the physiological functions, hence, resulted in better growth and yield. Very recently, it has been reported that PSB in combination with organic amendment i.e. pressmud improved the growth and yield of mash bean (Niazi *et al*., 2015).

Moreover, effective PSB isolate was identified as *Bacillus thuringiensis* strain S5 and it has been well reported that bacteria belonging to genus *Bacillus* mobilize the soil P through production of organic acids and phosphatase activity (Idris *et al*., 2002, 2004). Bacteria from genus *Bacillus* are globally used to formulate biofertilizers and biopesticides due to their ability to better survive in rhizosphere and multiple mechanism of plant growth promotion (Kumar *et al*., 2011).

## Conclusion

From this study, it can be concluded that application of multi-trait bacteria having phosphate solubilizing capability and phosphatase activity in interaction with poultry manure could be an effective approach for enhancing growth and yield of mung bean.

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