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Screening and characterization of rhizospheric phosphate solubilizing bacteria and their growth promoting effect on Mung bean *(Vigna radiata)*

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Abstract

Soil microorganisms are supportive in the transformation of soil phosphorus (P) and are thus an important component of the soil P cycle. Through their solubilizing and mineralizing abilities phosphate solubilizing microorganisms are effective in releasing P both from inorganic and organic pools of total soil P. To exploit this potential, the phosphate solubilizing bacteria were isolated from the rhizospheric soil. The six isolates were obtained in preliminary study. Out of six isolates two promising isolates [PSB-3 and PSB-6] were selected for study of metabolic diversity. The phosphate solubilization potential of PSB 3 was 17.34mg/l and PSB 6 was 19.68 mg/l. The plant growth promoting effect of PSB-3 and PSB-6 was assessed in pot study on Mungbean (*Vigna radiata)* by seed application, soil application and combination of seed and soil application in presence of 40 kg P_2O_5/ha. The parameters studied were germination time, number of leaves, shoot length and root length. The inoculation of isolates resulted in reduction in germination time, increase in number of leaves, increase in root length and shoot length. The isolate PSB 6 gave superior results as compared to isolate PSB 3. The combination of two isolates also gave beneficial effects. The study demonstrated that the use of selected isolates having multifaceted beneficial traits would be highly effective for improving growth and yield of crops.

Keywords: Phosphorus, phosphate solubilization, phosphate mobilizing bacteria, rhizospheric, Mungbean

Introduction

The world population is projected to reach at least 9.8 billion by 2050 according to the United Nation Food and Agriculture Organization (FAO) projections (Harold and Reetz, 2016). The global food security of this mammoth population will require at least doubling our current agricultural productivity (FAO, 2017, 2018, Soumare *et al*., 2020). For the fulfillment of this goal, it is necessary to have very fertile soils or alternatively supplement nutrients to soils having low fertility soils by applying a high amount of fertilizers (Keane, 2009). Till date, the chemical fertilizers have helped in feeding the world by providing three major plant nutrients, nitrogen, phosphorus and potassium (NPK) (Soumare *et al.*, 2020).

The Phosphorus (P) is the second most important macronutrient required by the plants, next to nitrogen. It is also one of the major growth-limiting macronutrients required for proper plant growth. It makes up about 0.2% and 0.8% of a plant's dry weight. The phosphorus (P) is important constituent of nucleic acids, enzymes, coenzymes, nucleotides, and phospholipids. P is essential in every aspect of plant growth and development, from the molecular level to

many physiological and biochemical plant activities including photosynthesis (Sharma *et al.,* 2013), development of roots, strengthening the stalks and stems, formation of flowers and seeds, crop maturity and quality of crop, energy production, storage and transfer reactions, root growth, cell division and enlargement, N fixation in legumes, resistance to plant diseases (Sharma *et al*., 2013, Kumar *et al*., 2018, Khan *et al.,* 2009., Satyaprakash *et al*., 2007), transformation of sugar to starch, and transporting of the genetic traits (Mehrvaz *et al*., 2008). Adequate P availability is also required for laying down the primordia of plant reproductive parts during the early phases of plant development (Satyaprakash *et al*., 2007, Kalayu, 2019).

On average, the phosphorus content of soil is about 0.05% (w/w); however, only 0.1% of this phosphorus is available for plant use (Zhu *et al*., 2011). Traditionally, the challenge of soil phosphorus deficiency is addressed by the application of phosphorus fertilizers. Approximately 52.3 billion tons of P-based fertilizers are applied annually to maintain available P levels in soil–plant systems (FAO, 2017). Whereas, only, about 0.2%, i.e.,<10µM of this huge amount, is used by plants (Alori *et al*., 2017; Islam *et al*., 2019) and the rest is converted inorganic P and precipitated by metal cations in soil such as Fe, Al, Mg, Ca, etc. (Soumare *et al*., 2020). These accumulated phosphates in agricultural soils are adequate to maintain maximum crop yields worldwide for about 100 years (Kalayu, 2019). Additionally excessive use of fertilizers poses various environmental problems such as, groundwater contamination and water way eutrophication (Yu, *et al*., 2011, Alori *et al*., 2017). Developing agricultural management strategies for improving phosphorus fertilization efficiency, increasing crop productivity and reducing environmental pollution caused by phosphorus loss from the soil is the need of time.

Soil microorganisms are involved in a wide range of biological processes including the transformation of insoluble soil nutrients, they also enhance plant nutrient acquisition (Glick, 2012). Soil microorganisms are capable of solubilizing and mineralizing insoluble soil phosphorus for the growth of plants. Apart from chemical fertilization, microbial P-solubilization and mineralization is the only possible way to increase plant available phosphorus. In the natural environment numerous microorganisms in the soil and rhizosphere are effective at releasing phosphorus from total soil phosphorus through solubilization and mineralization (Bhattacharyya and Jha, 2012, Alori *et al*., 2017). This group of microorganisms are referred to as Phosphorus Solubilizing Microorganisms (PSM). The use of efficient PSM is an one stop solution for improving P uptake by plants, increasing productivity and utilizing accumulated phosphates in agricultural soils.

In this context the present study was taken up to isolate PSM from rhizospheric soils and their efficacy as biofertilizer was tested in pot study on Mung bean (*Vigna radiata*).

Materials and Methods

Collection of Samples

The rhizospheric soil samples were collected from farms around Chhatral village of Kadi. Total 5 samples were collected from different farms and were immediately transported to laboratory for further studies.

Adaptation and Enrichment

The collected soil samples were adapted with 2% insoluble phosphate source (Tri calcium phosphate), and incubated for 1week at room temperature. After adaptation, enrichment of phosphate solubilisers was carried out by inoculating 1g soil in 100 ml Pikovaskaya's broth (Glucose 1%, $MgSO_4.7H_2O$ 1%, $CaCl_2$1%, Tri Calcium Phosphate 0.5%, pH 7.0) and incubating at 37^0 C on 120 rpm for 1 week.

Isolation and Screening of Phosphate Solubilizers

Isolation of phosphate solubilising bacteria was carried out from enriched samples using Pikovaskaya's agar and incubation at 37 for 1 week. (Pikovaskaya, 1948). For isolation of phosphate solubilising fungi Pikovskayas medium with pH-4.0-4.5 was used and incubation was carried out at 30 for 1 week. Colonies exhibiting clear zone of Phosphate solubilization were selected as Phosphate solubilizers and were isolated and purified for further study.

Phosphate solubilizing efficiency of all selected isolates was assessed by Khandeparkar's selection ratio method (Gayal and Khandeparkar, 1979).

Ratio = D/d = Diameter of zone of clearance/Diameter of growth

Acid production of selected isolates was also checked by using Pikovskaya's medium supplemented with bromothymol blue (pH indicator dye).

Characterization of Phosphate Solubilizing Bacterial Isolates:

The selected bacterial isolates were characterized culturally and microscopically. The metabolic diversity of selected bacterial isolates was studied using routine biochemical test and enzyme production ability.

Quantitative Estimation of P Released from Tri Calcium Phosphate (TCP)

Selected isolates were further examined for their ability to release P from TCP in liquid medium. One ml of 24 hours old culture (10^8 cells) was inoculated to 50 ml of Pikovaskaya's broth (Pikovaskaya, 1948). The inoculated flasks were incubated for three weeks at 28 ± 2 ^{0}C. The amount of P released in the broth was estimated from supernatant after centrifuging at 5000 rpm for 10 minutes on 7, 15 and 20 days of incubation. The available P content in the supernatant was estimated by phosphomolybdic blue color method of Ammonium molybdate (Muhr, *et al*., 1965).

Effect of Phosphate Solubilizing Bacteria on Growth of Mung bean (*Vigna radiata*)

A pot culture experiment was conducted using two efficient phosphorus solubilizing bacteria: One is PSB-3 and other is PSB-6. The experimental design for pot study experiment is shown in Table – 1.

Table 1: Experimental Design of Pot Culture Study

Experiment design	CRD (Complete Randomized Design)
Number of replicates	3
Treatments	6
Crop	Mung bean (*Vigna radiata*)
Pot size	7cm
Number of seeds/Pot	3
Fertilizer dose	Recommended dose N:P:K=40:40:20
Date of Sowing	29th January, 2019
Crop Duration	30 Days
Date of Harvesting	27th February, 2019

Pot experiment

Experiments for studying the effect of the phosphate solubilizing microorganisms on plant growth and P uptake of Mung bean was conducted using pot study. Three methods of application i.e Seed application, Soil application and Seed +soil application were carried out.

Experiment A: Seed application- seeds were treated with microbial inoculants for 30 minutes before sowing.

Experiment B: Soil application - microbial inoculants were inoculated in soil.

Experiment C: Seed + Soil application- seeds were treated with microbial inoculants for 30 minutes before sowing and microbial inoculants were inoculated in soil.

The experimental design is mentioned in Table – 2 to 4.

Table-2 Experiments-A: Seed Application of P Solubilizing Bacteria

Treatment no.	Chemical Fertilizer P_2O_5 kg/ha	Rock Phosphate P_2O_5 kg/ha	Seed Application of PSB
T1	-	-	-
T2	40	-	-
T3	20	20	-
T4	20	20	PSB-3
T5	20	20	PSB-6
T6	20	20	PSB-3 + PSB-6

Fertilizer dose : All treatments (T2-T6) except absolute control (T1) received 40 kg P_2O_5 / ha.
Seed Application : PSB-3 → 3 ml containing (10^8 CFU)/ kg seed before sowing.
PSB-6 → 3 ml containing (10^8CFU) / kg seed before sowing.
Irrigation : As and when needed.

Table-3 Experiment-B: Soil Application of P Solubilizing Bacteria.

Treatment no.	Chemical Fertilizer P_2O_5 kg/ha	Rock Phosphate P_2O_5 kg/ha	Soil Application of PSB
T1	-	-	-
T2	40	-	-
T3	20	20	-
T4	20	20	PSB-3
T5	20	20	PSB-6
T6	20	20	PSB-3 + PSB-6

Fertilizer dose : All treatments (T2-T6) except absolute control (T1) received 40 kg P_2O_5 / ha.
Soil Application : PSB-3 → Apply @ 3ml containing 10^8 CFU/ pot
PSB-6 → Apply @3ml containing 10^8 CFU/pot
Irrigation : As and when needed.

Table-4 Experiment-C: Soil +Seed Application of P Solubilizing Bacteria

Treatment no.	Chemical Fertilizer P_2O_5 kg/ha	Rock Phosphate P_2O_5 kg/ha	Seed Application	Soil Application
T1	-	-	-	-
T2	40	-	-	-
T3	20	20	-	-
T4	20	20	PSB-3	PSB-3
T5	20	20	PSB-6	PSB-6
T6	20	20	PSB-3 + PSB-6	PSB-3 + PSB-6

Fertilizer dose : All treatments (T2-T6) except absolute control (T1) received 40 kg P_2O_5 / ha.
Seed Application : PSB-3 → 3 ml containing10^8 CFU/ kg seed before sowing.
PSB-6 → 3 ml containing10^8 CFU/ kg seed before sowing.
Soil Application : PSB-3 → Apply @ 3ml containing 10^8 CFU/ pot
PSB-6 → Apply @ 3ml containing 10^8 CFU/ pot
Irrigation : As and when needed.

The insoluble and soluble phosphate was mixed thoroughly with the soil in a plastic bag before use. Three pots were used for each treatment. Three seeds were placed in each pot at a 1 cm depth.

Inoculum Preparation

Selected phosphate solubilizers were inoculated in nutrient broth and actively growing culture with density of 10^8 CFU/ml was obtained after 24 hour incubation.

Sowing and Maintenance

The inoculated seeds were sown in pots @ 3 seeds per pot in triplicates. After germination, thinning was done to retain one plant in each pot. The pots were watered regularly to maintain optimum moisture and other routine care was taken to protect the plants from pest and diseases.

Observation

Observations on plant growth parameters of *Vigna radiata* were recorded at 5, 10, 15 and 30 days after sowing (DAS). Seed germination time period required for the seeds were recorded in 5 days after sowing. Number of leaves per plant were counted and recorded at 10, 15, and 30 DAS. Root length was recorded at 30 DAS by uprooting the plant and measuring the length from tip of the longest root to the neck region and expressed in centimeters. Similarly the shoot length was measured from top of the tip to the base of plants at the surface of soil and expressed in cm.

Results and Discussion

Isolation and Screening

On the basis of the colony characterization, a total of 6 bacterial cultures were isolated from the collected soil samples. Morphologically distinct isolated colonies exhibiting cleared zone of phosphate solubilization on pikovaskaya's agar were selected. The total 6 bacterial isolates were obtained as phosphate solubilizers were coded as PSB-1 to PSB-6.

The PVK medium was used in the present study because it acts as specific isolation medium for phosphate solubilizing bacteria due to the presence of tricalcium phosphate, it is known for halo zone formation (Sharma, K., G. Dak and *et al*. 2007). Gaind (1987) reported that the PSB strains were isolated using the Pikovskaya's medium based on the formation of halo zone around these microorganisms. These findings were also supported by Ahamad & Jha (1977) that the phosphobacteria were identified by noting the solubilizing zone formed around the bacterial colony.

Based on the Khandeparkar's selection ratio, among the 6 bacterial isolates, 2 isolates exhibited the higher phosphate solubilization (i.e.PSB-3 and PSB-6) were selected for further studies (Fig-1). The Khandeparkar's ratio values for all the bacterial isolates is shown in table-5.

Table-5: Phosphate Solubilization Values of Isolates by Khandeparker's Selection Ratio.

Isolates	Diameter of zone of clearance (D) mm	Diameter of growth (d) mm	D/d (ratio)
PSB-1	8.5	7.0	1.2
PSB-2	10	7.5	1.3
PSB-3	**15.0**	**8.0**	**1.8**
PSB-4	13.0	9.0	1.4
PSB-5	7.2	6.9	1.0
PSB-6	**6.7**	**2.8**	**2.3**

All the isolates show solubilization mechanism through acid production when tested by Bromothymol blue containing agar medium (Fig. 2).

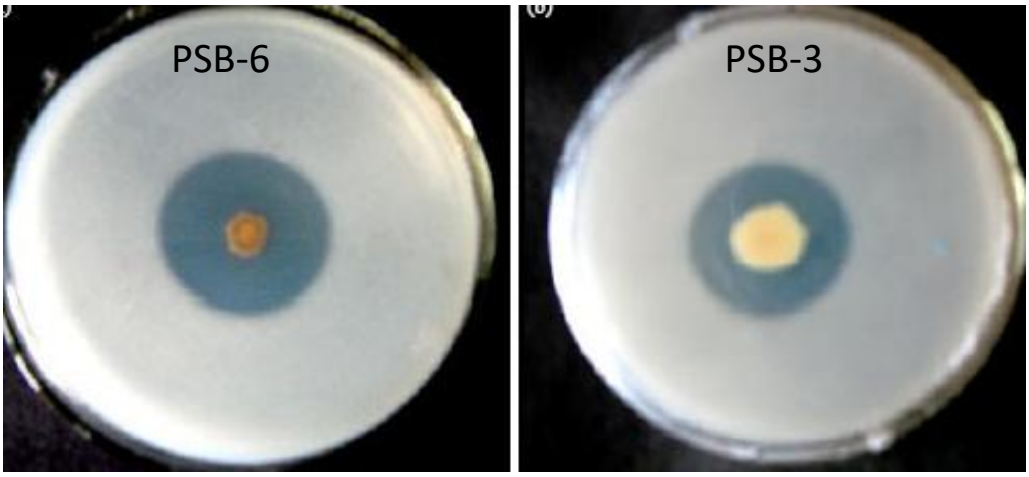

Fig-1: Zone of Tri Calcium Phosphate Solubilization by Bacterial Isolates on Pikovaskaya's Agar medium.

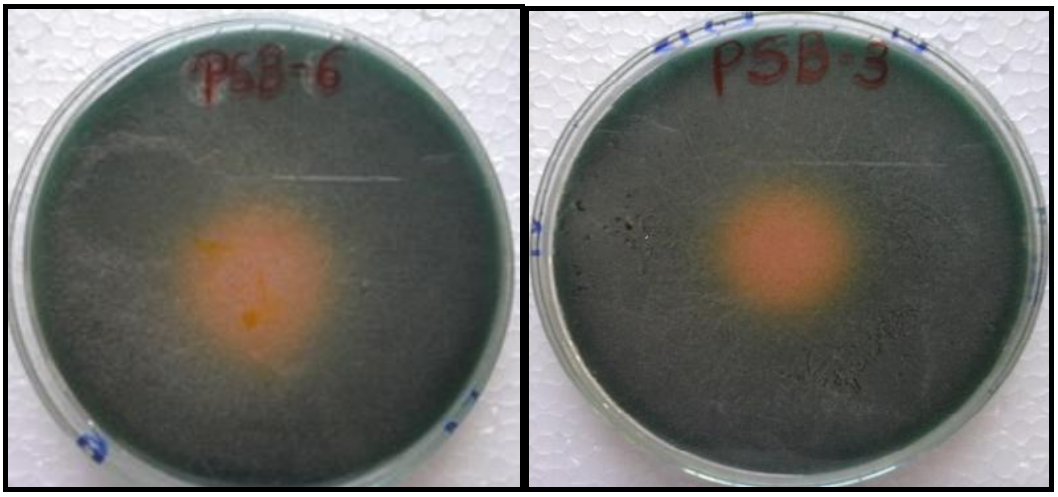

Fig-2: Acid Production by Bacterial Isolates on Pikovaskaya's Agar + BTB Medium.

Cultural and Morphological Characteristics

The morphological characteristics of the selected phosphate solubilizers are presented in Tables 6. PSB-6 was found to be Gram positive spore bearing rod while PSB-3 was found to be gram negative short rod with EPS production.

Table-6: Colonical, Morphological and biochemical characteristics of best Zinc mobilizing bacterial isolates.

S.N.	Bacterial Isolates	Cultural Characters	Gram reaction & Cell shape	Spore Formation	Capsule formation
1.	PSB-3	Small, White, Smooth, Raised	Gram Negative, Short rod	Non spore former	Non capsulated
2,	PSB-6	Medium, Light brown Dry, Raised	Gram Positive, Bacillus	Spore former	Non capsulated

Metabolic Diversity of selected Phosphate Solubilizing Isolates.

Metabolic diversity of PSB-3 and PSB 6 was studied in terms of various enzyme production (Table-7) and carbohydrates utilization (Table-8).

Table-7.Enzyme production ability of PSB 3 and PSB 6

Enzyme Production Test	Bacterial Isolates	
	PSB-3	PSB-6
Amylase	+	+
Protease	+	+
Lipase	+	+
Urease	+	-
Catalase	-	-
Phenylalanine deaminase	-	-
Nitrate reductase	-	+
Gelatinase	+	+

+ = Reaction positive / Enzyme produced,
- = Reaction negative / Enzyme not produced

Both Gram positive and Gram negative bacterial isolates were found to be facultative anaerobes and negative for phenylalanine deaminase test. Both organisms produced many enzymes like amylase, protease, lipase, gelatinase etc, indicating their ability to utilize diverse substrates. It is well known that enzymes plays a key role in transformation, recycling and availability of plant nutrients in soil. They are likely to be influenced by fertilizer and manures. Various enzyme activities were found to be maximum in treatment receiving PSB. In soil complex biochemical reactions used to occur regularly. Several enzymes in soil catalyze these biochemical reactions which are responsible for nutrient cycling in soils.

Carbohydrate Utilization by the Phosphate Solubilizing Bacterial Isolates

The metabolic diversity of PSB 3 and PSB 6 was further assessed by testing their ability to utilize 36 different carbon sources. The isolate PSB-3 utilize 9 different carbon sources while PSB-6 utilize 13 different carbon sources. The form of available carbon sources greatly affected the growth as well as the phosphate solubilization which was more active in presence of hexoses and pentoses or dissacharides (Patil *et al*. 2001).

Table-8. Various carbohydrates utilization Phosphate Solubilizing Isolates.

Sugar Utilization	PSB-3	PSB-6	Sugar Utilization	PSB-3	PSB-6	Sugar Utilization	PSB-3	PSB-6
Lactose	-	-	Insulin	-	+	Rhamnose	-	-
Xylose	-	-	Sodium gluconate	-	+	Cellobiose	-	+
Maltose	+	+	Glycerol	+	+	Melezitose	-	-
Fructose	+	+	Salicin	-	-	A-Methyl-D-mannoside	-	-
Dextrose	+	-	Dulcitol	-	-	Xylitol	-	-
Galactose	-	-	Inositol	-	-	ONPG	-	+
Raffinose	-	-	Sorbitol	-	-	Esculin hydrolysis	+	-
Trehalose	+	-	Mnnitol	-	+	D-Arabinose	-	+
Melibiose	-	-	Adonitol	-	-	Citrate	+	+
Sucrose	+	+	Arabitol	-	-	Malonate	+	+
L-Arabinose	-	-	Erythritol	-	-	Sorbose	-	-
Mannose	-	+	α-Methyl-D-glucose	-	-	Rhamnose		

+ = Reaction positive / sugar utilized
- = Reaction negative / sugar utilized

Quantitative Estimation of Pi Released from Tri Calcium Phosphate

The amount of Pi released from TCP in the Pikovaskaya's broth by two PSB isolates was studied at 7, 15, and 20 days after incubation (DAI). The amount of Pi released from TCP by all bacterial isolates increased with advanced incubation time. The isolates PSB 6 gave 19.68 mg/lit phosphate solubilization and isolate PSB 3 gave 17.34 mg/lit phosphate solubilization(Fig-3). Amongst both PSB-6 was superior as compare to PSB-3 but at 15 DAI PSB 3 was better solubilizer as compare to PSB 6. *In vitro* Pi released by PSB isolates from TCP ranged from 17.47 to 21.5 per cent (Bardiya, 1970; Pal *et al.,* 2004).

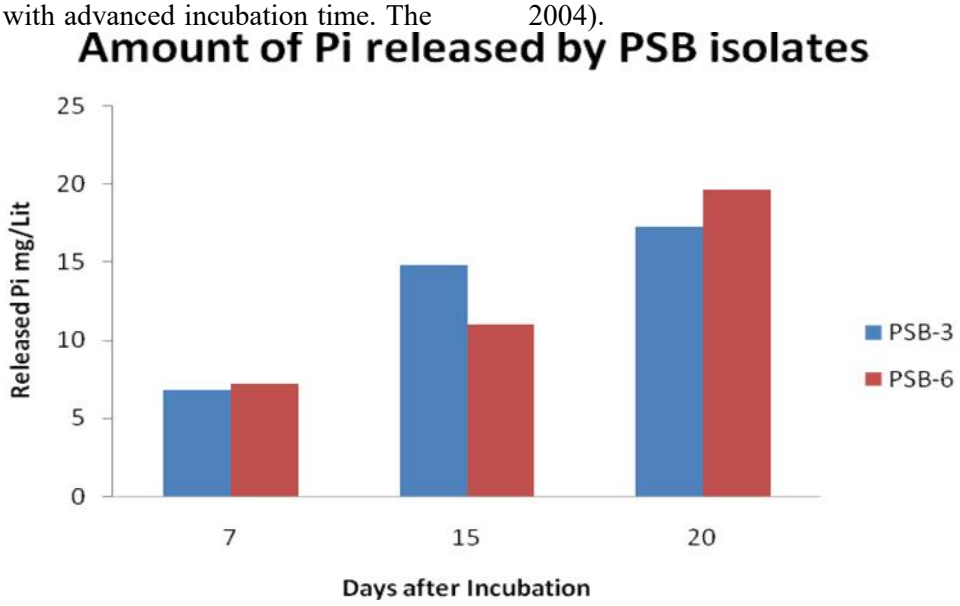

Fig. 3 Amount of phosphate released by isolates

Effect of Phosphate Solubilizing Bacteria on Growth of Mung bean (*Vigna radiata*)

The effect of inoculation of 2 selected phosphate solubilizing isolates on growth parameters of Mung bean was studied by pot experiment The results were recorded at the end of 5, 10, 15, 20 and 30 DAS in terms of seed germination time, number of leaves, root length and shoot length (Table-9 and 10).

Effect of PSB on Seed Germination

There was significant difference observed in the days required for the seed germination when the Mung bean plant was inoculated with the P solubilizing isolates. In uninoculated seed the germination time was approximately 6-7 days whereas all the seeds inoculated with PSB showed germination time of 3-5 days (Fernandez *et al.,* 2007). There was considerable reduction in germination time after treatment with PSB however the best combination was seed + soil inoculation with PSB where germination time reduced to 50% as compared to control (Table-9).

Effect of PSB on Number of Leaves per Plant

The PSB inoculation had a positive impact on number of leaves per plant. The number of leaves approximately doubled in plants inoculated with PSB as compare to uninoculated plant. The best results obtained in the treatment of soil plus seed application and seed application as compared to soil application (Table-9).

Table-9. Effect of PSB-3 and PSB-6 isolates on Seed Germination and Number of Leaves to Mung bean Plant. The values are the mean of three replications (±SD).

S. N.	Treatments	Seed Germination (Days taken) (Mean± SD)			Number of Leaves per Plant at 15 DAS (Mean± SD) (in cm)		
		Exp A	Exp B	Exp C	Exp A	Exp B	Exp C
1	Uninoculated	6.6 ±0.47	6 ±0	6.6 ± 0.47	3.3 ± 0.94	3.6 ± 0.7	3.0± 0
2	Uninoculated + soluble P	6 ± 0	5.6 ± 0.47	5.3 ± 0.47	3.6 ± 0.47	3.6 ± 0.47	4.0 ± 0.81
3	Uninoculated +Soluble P + Rock P	6 ± 0.81	5± 0.81	4.6 ± 0.47	4 ± 0.81	4.5 ± 0.81	4.6 ± 0.47
4	Soluble P + Rock P + PSB-3	4.3 ± 0.47	4.3 ± 0.47	4 ± 0.81	4.6 ± 0.47	5.6 ± 1.24	6 ± 0.81
5	Soluble P + Rock P + PSB-7	4.3 ± 0.47	3.6 ± 0.94	3.3 ± 0.47	5.6 ± 0.47	6.3 ± 0.47	5.3 ± 0.47
6	Soluble P + Rock P + PSB-3 + PSB-6	4.3 ± 0.47	4 ± 0.81	3.3 ± 0.47	6.3 ± 0.47	6 ± 0.81	6.3 ± 0.47

Effect of PSB on Shoot and Root length

A significant increase was also observed in the shoot and root length of the Mung bean plant treated with phosphate solubilizing bacteria. Seed plus soil application gave superior results as compare to all the other treatments. Approximately 18 to 20% increase was observed in root and shoot length of plants treated with PSB (Table-10).

Table-10. Effect of PSB-3 and PSB-6 isolates on Shoot and Root Length of Mungbean Plant. The values are the mean of three replications (±SD).

S.N.	Treatments	Root Length (Mean ± SD) 30 DAS			Shoot Length (Mean± SD) 30 DAS (in cm)		
		Exp A	Exp B	Exp C	Exp A	Exp B	Exp C
1	Uninoculated	5.3 ± 0.26	5.1 ± 0.09	5.5 ± 0.09	9.7 ± 0.20	10 ± 0.14	9.7 ± 0.12
2	Uninoculated + soluble P	5.6 ± 0.20	5.6 ± 0.04	5.4 ± 0.16	10.1 ± 0.08	10.2 ± 0.09	10.2 ± 0.30
3	Uninoculated+ Soluble P + Rock P	5.4 ± 0.16	5.6 ± 0.16	5.8 ± 0.08	10.4 ± 0.09	10.5 ± 0.12	10.4 ± 0.46
4	Soluble P + Rock P + PSB-3	5.9 ± 0.26	6.3 ± 0.18	6.3 ± 0.09	10.7 ± 0.18	10.1 ± 0.09	11 ± 0.14
5	Soluble P + Rock P + PSB-6	6.4 ± 0.16	6.8 ± 0.14	6.5 ± 0.16	10.9 ± 0.12	11.2 ± 0.24	10.9 ± 0.16
6	Soluble P + Rock P + PSB-3 + PSB-6	6.4 ± 0.26	6.7 ± 0.12	6.6 ± 0.12	11.5 ± 0.16	11.3 ± 0.20	11.4 ± 0.12

Two efficient PSB strains *(P. agglomerans* PSB-1 and *B. anthina* PSB-2) had a marked insoluble phosphate solubilizing ability as visualized by the clear zone developed around the colonie (Walpoa and Yoon, 2013). Few reports also described some *Burkholderia* and *Pantoea* strains as being efficient phosphate solubilizers (Peix *et al.,* 2001a and b; Caballero-Mellado *et al.*, 2007; Torres *et al*., 2008; Viruel *et al*., 2011; Silini-Cherif *et al*., 2012).

In pot experiments of Mung bean, as compared to uninoculated and untreated soil (Control) the uninoculated plants treated with rock phosphate and soluble phosphate showed slight stimulatory effects on plant growth parameters. However the stimulatory effects were not very significant indicating that native soil flora and microorganisms were unable to mobilize the added insoluble phosphate to large extent. The treatments of plants with PSB in presence of insoluble phosphates gave best results in terms of plant growth parameters, highlighting the facts that added PSB-6 not only survive in the soil but also mobilize the added insoluble phosphate and improved the phosphate availability to plant. Increased growth and P uptake of several crop plants due to PSB inoculation have been reported in a number of studies conducted under both growth chamber and greenhouse conditions (Dey *et al*., 2004; Fernandez *et al*., 2007; Vikram and Hamzehzarghani, 2008; Hariprasad and Niranjana, 2009; Yu *et al*., 2011). The increase in shoot length, root length of Mung bean plants inoculated with PSB strains could be attributed to a greater absorption of nutrients, especially P. Results of the present study that, plant growth of Mung bean is stimulated with co-inoculation of two phosphate solubilizing bacterial isolates (PSB-3 and PSB-6) with TCP are in line with the findings of (Qureshi *et al*., 2011).

From the selected two isolates PSB-3 and PSB-6 the isolate PSB-6 gave superior results in terms of plant growth parameters in all treatments and applications. Compared with single inoculation, co-inoculation showed higher growth performances and P uptake; this suggests that both strains acted synergistically with each other to promote Mung bean plant growth. Also among the applications tested soil plus seed applications of co inoculation with PSB-3 and PSB-6 is the most promising mode of applications for Mung bean.

Conclusion

Total 6 phosphate solubilizing isolates were obtained by plate assay method from rhizospheric soil. Isolate PSB-3 and PSB-6 gave 17.47 to 19.58 mg/lit phosphate solubilization respectively. Both the isolates had capacity to produce diverse enzymes which could be instrumental in nutrient transformation in soil. The pot study results indicated PSB-6 as better phosphate solubilizer as compare to PSB-3. The seed plus soil inoculation is the best way for the applications of PSB. However co inoculation of both the isolates gave significant increase in plant growth parameters of Mung bean (*Vigna radiata*). The findings highlighting the synergistic and complimentary effect of combined inoculation of both the isolates and their probable use as plant growth promoters.

As the phosphate is a major plant growth limiting factor, addition of phosphate chemical fertilizer is a common practice to combat phosphate deficiency. However only 10 % of added fertilizer becomes available to plant and remaining phosphate is bound with other minerals and becomes unavailable to plants. Hence the use of phosphate solubilizing microorganism (PSM) is an effective strategy to mobilize these bound phosphates and make it available to plant. The application of PSM in long run will eliminate the dependence of chemical fertilizers and will promises a clean and better environment. Additional benefits also include production of plant growth promoters like IAA and GA, siderophores production, protection from pathogens, nitrogen fixation, K solubilization and improved plant growth and yield.

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