ISOLATION OF PHOSPHATE SOLUBILIZING BACTERIA FROM THE RHIZOSPHERIC SOIL OF TOMATO PLANT AND USED AS BIO- FERTILIZER ON MUNG BEAN PLANT.

MOHINI K. PATEL¹ & DR HEMANGI DESAI^{2*},

¹PG Scholar, Environment Science Department, Shree Ramakrishna Institute of Computer
Education and Applied Science, Surat, India

²Assistant Professor, Environment Science Department, Shree Ramakrishna Institute of Computer
Education and Applied Science, Surat, India

Abstract

Chemical phosphate fertilizer is the main source of available Phosphorous in the agriculture soil, but most of the phosphate is precipitated by iron, aluminium and calcium present in soil. It is unavailable for plant to consume. For that problem of phosphate deficiency are occurred. Plant available phosphate is present in soil as orthophosphate anions $H_2PO_4^{-1}$ and $H_2PO_4^{-2}$. Inorganic and organic P are poorly soluble in soil. Phosphate solubilizing bacteria (PSB) produce a variety of organic acids including acetic, gluconic, formic, propionic acids that covert the insoluble P into soluble P. PSB hydrolyze the organic P in form of organic residues by production of acid phosphatases enzymes. PSBs include various genera like *Aspergillus*, *Penicillium*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Enterobacter* and *Azotobacter*. These PSBs are isolated on the agar media contain tricalcium phosphate. Among these bacteria *Rhizobium* and *Azotobacter* are helped to fix nitrogen and also produce IAA (Indole acetic acid) which enhances root proliferation and growth of crop plant. PSBs are used as biofertilizer in agricultural crops.

Keywords: PSBs, Rhizospheric soil, IAA, Enzyme, Biofertilizer.

Original Article

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1. INTRODUCTION

Overview of PSB (Phosphate Solubilizing Bacteria) and Soil Phosphorous

Phosphorous is the one of the major nutrient for the growth as the role in the cell division, nuclear formation and essential for man metabolic processes of the plants. Phosphorous are found in abundant in both organic and inorganic form (soil). There are three form of phosphorous; orthophosphate, polyphosphate and organic phosphate. Plant takes up soluble form of phosphate along with water to fulfil the requirement of phosphorous. Whereas insoluble form are not available for the plants, as it form the insoluble form with calcium in alkaline soil, whereas iron and aluminium in acidic soil that leads to precipitation. Many microorganisms to be found, which solubilized insoluble form of phosphate and increase the available form of phosphate to plant, through the production of organic acid (acidification), production of chelating ion (chelation) and exchange reaction. Bacteria and fungi are the most reported to solubilized the inorganic form of phosphate.

In this study involving plants inoculated with organism showed the growth enhancement and increase the

phosphate content through the PSM effects phosphate solubilizing microorganism (PSM) are ubiquitous and their number are various from soil to soil. Bacteria have high efficiency to solubilized inorganic form followed by fungi, actinomycetes and yeast. Some nitrogen fixer species were reported to solubilized insoluble form of phosphate. Phosphate solubilizing organisms are widely used as a biofertilizer for the field application on different crops to enhancement of growth and production and also play a role as a biocontrol agent. Phosphate solubilizing microorganisms (PSM) include largely bacteria and fungi, which can grow in media containing tricalcium, iron and aluminium phosphate, hydroxyapatite, bonemeal, rock phosphate and similar insoluble compounds as the sole phosphate source. The most efficient PSM belong to genera *Bacillus* and *pseudomonas* amongst bacteria and *Aspergillus* and *penicillium* amongst fungi. However the application of the biofertilizer formulated using bacteria has wide application compare to fungi biofertilizer, as it requires the high humidity and cannot tolerant the elevated salinity and temperature. Whereas the bacteria efficiently grow in the presence of adverse condition.

Phosphorous in Soil and its Role in Plant Nutrition

Next to Nitrogen, Phosphorus (P) is the second most important macro-nutrient required by the plants. Unlike for nitrogen there is no large atmospheric source that can be made biologically available for P availability (Mc Vickar *et al.*, 1963). Phosphorus occurs in fully oxidized state as phosphate, but invariably forms a large number of insoluble chemical complexes with calcium, iron and aluminium, forming insoluble phosphate salts present in the soil, which indeed makes this nutrient a paradox. It is reported to be a critical factor of many crop production systems, due to the fact that the limited availability in soluble forms in the soils (Khan *et al.*, 2007). Phosphorus is the least mobile nutrient element in plant and soil compared to other essential macronutrients (Gyaneshwar, 2012). Soil P dynamics characterized by physico-chemical, sorption-desorption and biological, immobilization-mineralization (Mc Vickar, 1963). P either by adsorption or chemical precipitation becomes immobilise or less soluble in soil (Gyaneshwar, 2012). The concentration of P in soil solution is very low, varying from 0.001mg L⁻¹ in very poor soils to 1mg L⁻¹ in heavily fertilized soils. In India about 98% of soils including fertile ones are deficient in P, as the concentration of free P (available form for plants) .

The form of phosphate taken up by the plant from soil solution is phosphate anions mainly H₂PO₄⁻ and HPO₄²⁻ (Mc Vickar, 1963). Large amounts of P applied as P fertilizers get immobilized by the active and rapid chemical reactions with cations such as Ca²⁺ in calcareous or normal soils to form a complex Calcium Phosphate (Ca₃ (PO₄)₂) and with Al³⁺ and Fe³⁺ in acidic soils to form Aluminium Phosphate (AlPO₄) and Ferrous Phosphate (FePO₄) which are sparingly soluble precipitates (Vassileva *et al.*, 2001) and ultimately resulting in the low concentration of P in soils making it unavailable to plants. Therefore, crop plants can utilise only a fraction of applied P and this make them poor in performance (Seema *et al.*, 2013). For example, P deficiency in legumes can severely affect plant growth and productivity where both plant and its symbiotic bacteria are affected posing deleterious effects on nodule formation and function. Release of this insoluble, fixed or adsorbed form of P by phosphate solubilising microorganisms(PSM) is an important aspect of P availability in soils (Khosro, 2012).

Inorganic forms of P are solubilized by a group of heterotrophic microorganisms excreting organic acids that dissolve phosphatic minerals and / or chelate cationic partners of the P ions directly releasing P into solution (He *et al.*, 2002). Plant growth promoting (PGP) microorganisms, including PGP-Rhizobacteria (PGPR), phosphate mycorrhizas (AM) fungi, may play a major role in developing a constituent element of nucleic acids, enzymes, coenzymes, nucleotides and phospholipids, it is involved in the transformation of energy, transfer of hereditary characters and cell organization in

plants (Arnou, 1953). The overall contribution associated with P nutrition to plants include, root development, stalk and stem strength and growth, formation of flower and seed, resistance to plant diseases, crop quality, maturity and production. N fixation is the major task associated with P nutrition in legumes (Deepshikha *et al.*, 2014). It is also involved in photosynthesis, macromolecular biosynthesis and respiration (Khan *et al.*, 2010) during the early phases of plant development .

Phosphate solubilizing bacteria are belongs to the PGPR. The term Plant Growth Promoting Rhizobacteria was first defined by Kloepper and Schroth in 1978 as soil bacteria that colonize the rhizosphere of plants, and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere (Kloepper *et al.*, 2003). Rhizospheric microorganisms mediate soil processes such as soluble compounds exudation, storage and release of nutrients and water, nutrient mobilization and mineralization by roots and microorganisms, soil organic matter decomposition, phosphate and potassium solubilization and nitrogen fixation, nitrification, denitrification and sulphur reduction (Flaishman *et al.*, 1996; Deubel *et al.*, 2000).

Phosphate Solubilising Bacteria

Figure Movement of phosphorous in soil (Ahemad and Kibret, 2014)

A variety number of microbial species have the capacity to solubilise P. These include bacteria, fungi, actinomycetes and algae and are collectively called as Phosphate Solubilising Microorganisms (Hani, 2012). Bacteria are more effective in P solubilization than fungi (Alam *et al.*, 2002). PSB are ubiquitous and varies in shape and population in different soils. Their population in soil depends on chemical and physical properties, organic matter and P content of the soil (Kim, 1998). PSB were found in majority of soils (Chhonker and Taraedar, 1984). Their population was generally low in arid and semi-arid regions, possibly due to the low level of organic matter and high temperature regime (Gupta *et al.*, 1986). The PSB population was higher in soils under mild and moist climates than in dry ones (Subba Rao, 1982). Agricultural and rangeland soils are found with larger populations of PSB (Yahya and Azawi, 1998). Among the whole microbial population in soil, phosphate solubilizing bacteria (PSB) constitute 1 to 50%, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5% in P solubilization have the ability to produce plant growth promoters.

The solubilization of insoluble phosphates in the rhizosphere is one of the most common mode of action of plant growth promoting bacteria (PGPB) that enhance nutrient availability to plants (Richardson *et al.*, 2001). The PSB may release several organic acids including oxalic, citric, butyric, malonic, lactic, succinic, malic, gluconic, acetic, glyconic, fumaric, adipic, and 2-ketogluconic acid (Rose, 1957). Salstorm in 1903 first demonstrated the microbial solubilization of

inorganic phosphates by incubating TCP (Tricalcium phosphate) with bacteria from milk and soil infusions as mentioned by Deepshika *et al.*, (2014) and Gerretson in 1948 demonstrated the microbial activity in the rhizosphere could dissolve the sparingly soluble inorganic P and increase plant growth, mentioned the same (Deepshika *et al.*, 2014).

Different bacterial genera and within genera different bacterial species have been reported to have P solubilising capacity. Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* (Whitelaw, 2000), *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, and *Enterobacter* are the most powerful P solubilizers (Subbarao, 1988). *Acetobacter* sp. (Joseph and Jisha, 2009), *Acetobacter diazotrophicus* (Maheshkumar *et al.*, 1999), (Rodriguez and Fraga, 1999), *Agrobacterium* sp. and *Alcaligenes* sp., *Corynebacterium* sp. (Gupta *et al.*, 1998), *Azotobacter chroococcum* (Kumar and Narula, 1999), *Burkholderia* sp., *Gluconacetobacter* sp., *Enterobacter* sp. (Chung *et al.*, 2005; Kim *et al.*, 2003), *Flavobacterium* sp. *Micrococcus* sp. (Goldstein, 2001), *Pseudomonas*, *Bacillus*, *Rhizobium*, *Micrococcus*, *Flavobacterium*, *Achromobacter*, *Erwinia*, *Acinetobacter* sp. and *Agrobacterium* (Rodriguez and Fraga, 1999) are among the frequently reported PSB. Although fungi are also known to solubilize P, the majority of the role is played by PSB in solubilising inorganic P.

The principal mechanism in soil for solubilization of P is lowering of soil pH by microbial production of organic acids or the release of protons and mineralization by producing acid phosphatases (Tarafdar and Claasen, 1988) ultimately resulting in P availability in soil (White law, 2000; Khan *et al.*, 2007; Khosro, 2012). Soil phosphates mainly of phosphatic fertilizers under alkaline conditions are fixed in the form of insoluble phosphates. Many of the calcium phosphates, including rock phosphate ores (fluoroapatite, francolite), are insoluble in soil. Their solubility increases with a decrease of soil PH. Phosphate solubilization is the result of combined effect of pH decrease and organic acids production (Fankem *et al.*, 2006). PSB decrease the soil pH by producing organic acids. These organic acids compete with the P binding sites in the soil (Nahas, 1996). Through Organic acid production PSB mobilizes P from sparingly soluble phosphates and convert into soluble forms mainly by the chelation mediated mechanism (Whitelaw, 2000). Acidification of the surroundings of microbial cell releases proton through the production of organic acids (Villegas and Fortin, 2002). These metabolite organic acids as a result of anion exchange of P, by an organic acid anion can either directly dissolve the mineral P or can chelate Al^{3+} , Fe^{3+} , Ca^{2+} -ions associated with inorganic P. The carboxyl and hydroxyl groups are chelate the cation that bound to inorganic P and convert it into soluble forms (Sagoe, 1998; Seema *et al.*, 2013) with the net result of an enhanced availability of it to the plant. As the soil pH increases the divalent and trivalent forms of inorganic P, HPO_4^{-2} and HPO_4^{-3} occur in the soil. The organic acids released by PSB into the soil consequently decrease the soil pH and acidifies the surrounding environment.

The type of organic acid produced and their amounts differ with different organisms. Among them, gluconic acid and 2-ketogluconic acid seems to be the most frequent agent of mineral phosphate solubilization (Deubel *et al.*, 2000; Song *et al.*, 2008). Other organic acids, such as acetic, citric, lactic, propionic, glycolic, oxalic, malonic, succinic acid, fumaric, tartaric etc., have also been identified among phosphate solubilizers (Ahmed and Shahab, 2011). These organic acids are the products of the microbial metabolism, mostly by oxidative respiration or by fermentation of organic carbon sources like glucose (Trollove *et al.*, 2003).

PSB mineralize soil organic P by the production of acid phosphatases. Release of organic anions, and production of Siderophores and acid phosphatase enzyme by microbes and hydrolyze the soil organic P or split P from organic residues resulting in P availability (Dodor and Tabatabai, 2003). PSB are also produce plant growth promoter IAA (indole

acetic acid). Phosphate solubilizing bacteria also produce IAA (indole acetic acid). IAA is one of the most physiologically active auxins. IAA is the common product of L-tryptophan metabolized by bacteria. Organisms such as bacteria, fungi and algae are able to make physiologically active IAA that may have pronounced effects on plant growth. Bacteria isolated from the rhizosphere have the capacity to synthesize IAA in vitro in the presence or absence of physiological precursors, mainly tryptophan. Production of IAA by microbial isolates varies greatly among different species and strain and depends on the availability of substrate. Although in the presence of tryptophan microbes release greater quantities of IAA. IAA is the main auxin in plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity. IAA plays a key role in the regulation of plant growth and development.

2. METHODOLOGY

Soil Sample Collection

The soil sample collected from the rhizosphere (surrounding the roots) of the tomato plant roots, sample collected from the depth of 10-15cm and put it in to the polyethylene bag. Keep it into the laboratory refrigerator until its analysis.

Enrichment and Isolation of Phosphate Solubilizing Bacteria

Isolation was carried out by enrichment technique. Take 1gm of soil sample and put in into the 100ml Pikovskaya's broth. This pvks broth contains insoluble tricalcium phosphate as the sole phosphate source in this medium. Put the flask in to the incubator rotatory shaker at 30⁰c for 5-7 days. After the enrichment is carried out, streak the loopful of suspension on to the Pikovskaya's agar plate. And put into the incubator at 30⁰c for 5-7 days. After incubation the colonies were surrounded by a clear zone indicating the phosphate solubilization. The colonies were selected, subcultured on the same medium and screened for further identification.

Identification of Microbes

The isolated bacteria was identified by morphological characteristic in which gram staining, shapes, IMVIC test and motility including catalase, Oxidase test, glucose, maltose, mannitol, lactose fermentation, starch hydrolysis, gelatin hydrolysis and nitrate reduction.

Analysis of Phosphate Solubilizing Activity

From the isolates, larger halo zone producing strains were selected for further study. The qualitative as well as quantitative analysis of Phosphate solubilizing activity of the selected isolates were conducted by plate screening method and broth culture method.

Qualitative Method

All the suspected colonies were screened for phosphate solubilization on Pikovskaya's medium. Isolates showing phosphate solubilizing ability were spot inoculated at the centre Pikovskaya's s plate and incubated at 37⁰C. Diameter of clearance zone was measured successively after 24 hours, up to 7 days. The Phosphate

Solubilization Efficiency (PSE) is the ratio of total diameter .i.e. clearance zone including bacterial growth and the colony diameter.

$$PSI = \frac{\text{colony diameter} + \text{clearzone diameter}}{\text{colony diameter}}$$

All the observations were recorded in triplicate. Strains developing clear zones around their colonies could easily identify as PSM.

Quantitative Method

Pikovskaya's s broth medium (100 ml) with Tricalcium phosphate (0.3g/100ml) was prepared and sterilized; 1ml of each isolates was inoculated into the broth medium. Then the inoculated sample were incubated for 5 days on rotatory shaker 370C after incubation, culture broth was centrifuged at 10,000rpm for 30min. Uninoculated broth served as control. The available Phosphorous was determined using colourimetrically at 410nm with standard KH₂PO₄.

Production of Indole Acetic acid (IAA) by PSB:

The quantitative estimation of indole acetic acid in broth cultures were determined by a colorimetric assay technique using Salkowski reagent. The O.D was determined at 530 nm in a spectrophotometer. A standard curve was prepared from serial dilutions of IAA stock solution. 100 mg of indole acetic acid was dissolved in 100 ml distilled water by slight heating from this stock solution. Indole acetic acid standard solution was as prepared in different concentration. inoculating the isolates in the 100 ml nutrient broth medium contain 1% L-tryptophan incubate for 48 hour at 37°C in 120 rpm an incubator shaker after incubation cultures centrifuged at 10000 rpm for 15 minute . four different isolates inoculated in different four nutrient broth .(uninoculated act as control) 1 ml of supernant was pipetted out and 4 ml Salkowski reagent added keep it in dark for 30 minute . The formation of pink colour was observed and the colour change was measured by spectrophotometer at 530 nm.

Preparation of Inoculum

Four flask contain 100 ml nutrient broth was inoculated with four different culture from the Pikovskaya's agar slant and incubated at 30°C for 48 hours the cells were separated the broth by centrifugation at 10000 rpm for 20 minute . It was washed twice with sterile distilled water and re-suspended in distilled water. This suspension was used as an inoculum. All the steps were carried out in aseptic conditions.

Seed Germination

Autoclave five petri dishes contain cotton, wash mung bean seeds (10-15) with sterile distilled water and put these seeds on the petri dish contain cotton with help of forceps. take 1 ml of inoculum and 10 ml sterile distilled water in petri dish (in four different petri dish added four different isolated inoculum).In one petri dish inoculum is not added , only sterile distilled water is added which act as control. After 5-7 days seed germination is observed. After the germination occurs, the percentage of germination of the seeds was calculated by recording the total number of seeds germinated to the total number of seeds shown after 10 days. Plants were selected for measuring the shoot length and root length. The shoot length was measured from the collar region to tip of the shoot and expressed in centimetre. And root length was measured from the collar region to the tip of the longest root in centimetre.

The vigour index was calculated by using the following formula;

$$\text{Vigour index} = \% \text{ germination} \times \text{seedling length (shoot length + root length)}$$

Pot Experiment

In this pot experiment study, the soil which is used in pot is autoclave first, so any other micro organisms are present in the soil were killed. In this soil insoluble tricalcium phosphate is added as the sole phosphate source. the pot culture experiment were conducted to study the effect of PSM on the growth of mung bean plant .The four isolates were screen for their efficient plant growth promotion ability were used for pot culture studies . The inoculum is used as bio fertilizer in farm and pot in different ways like this. The inoculum is applied in soil by inoculating in seeds, in roots, in soil and compost. In this study the inoculum used as bio fertilizer in liquid medium and apply in seeds by the mixing of 1 ml of inoculum in 20 ml of sterile distilled water ,mixed it well and dry then put into the pot for the study (four different inoculum is added into the four different pot). In uninoculated pot only autoclave soil is used (inoculum is not added in control pot soil).

Analysis of Pot Soil Parameter (Initial and Final)

Determination of soil parameters (before and after plant growth) like PH, EC, Moisture contain, Organic carbon and total Phosphate as per standard procedure of APHA.

Growth of Isolates on their Selective Agar Medium

All different types of phosphate solubilizing bacteria are grown on their selective agar medium as per Rakesh Patel book composition.

3. RESULT AND DISCUSSION

Result of Morphological Characteristics of PSB

Isolate	Medium	Gram Reaction and Morphology	Figure
A	Pikovskaya's medium	Short Rods Single Pink Gram negative
B	Pikovskaya's medium	Short Rods Chain, single Purple Gram positive

C	Pikovskaya's medium	Short Rods Single Pink Gram negative
D	Pikovskaya's medium	Short Rods Single, cluster Pink Gram negative

Result of Colony Characteristics of PSB

Isolates	Media	Colony characteristics	Observation
A	Pikovskaya's medium	Size Shape Edge Elevation Texture Consistency Opacity Pigmentation	Large Rod Entire Convex Smooth Moist Opaque-
B	Pikovskaya's medium	Size Shape Edge Elevation Texture Consistency Opacity Pigmentation	Large Rod Entire Convex Smooth Moist Opaque -
C	Pikovskaya's medium	Size Shape Edge Elevation Texture Consistency Opacity Pigmentation	Large Rod Entire Convex Smooth Moist Opaque -
D	Pikovskaya's medium	Size Shape Edge Elevation Texture Consistency Opacity Pigmentation	Large Rod Entire Convex Smooth Moist Opaque -

Result of Bio Chemical Characteristics of the PSB

Sr. no	Characteristics	Isolate A	Isolate B	Isolate C	Isolate D
1	Indole	-	-	-	+
2	Methyl red	-	-	-	-
3	Vogues proskauer	-	-	-	-
4	Citrate utilization	+	+	-	+
5	Urease	-	+	+	+
6	Nitrate reduction	+	-	+	+
7	H ₂ S production	-	-	-	-
8	Gelatine hydrolysis	+	+	-	-
9	Starch hydrolysis	-	+	+	+
10	Oxidase test	+	+	+	+
11	Catalase test	+	+	+	+
12	Glucose fermentation	+	+	+	+
13	Lactose fermentation	-	+	+	+
14	Maltose fermentation	-	+	+	+
15	Mannitol fermentation	+	+	+	+

*A= *Pseudomonas*, B= *Bacillus*, R = *Rhizobium*, D= *Azotobacter*

Result Qualitative Analysis of PSB

Table of Qualitative Analysis of PSB

Sr. No	Isolates	Colony diameter (cm)	Zone diameter(cm)	Solubilizing index(SI)
1	A	0.9	1.2	2.33
2	B	1.1	1.4	2.27
3	C	0.8	0.4	2.22
4	D	0.7	0.4	1.57

SI = (colony diameter + zone diameter) / colony diameter

Result of Quantitative Analysis of Phosphate Solubilizing Bacteria

Phosphate Solubilizing Activity of four different Phosphate Solubilizing Bacteria

S. No	Isolates of PSB	Soluble Phosphate Concentration(mg/l)
1	Isolate-A(Pseudomonas)	2.3
2	Isolate-B(Bacillus)	1.9
3	Isolate-C(Rhizobium)	0.5
4	Isolate-D(Azotobacter)	0.7

Result of IAA Production Test

Result of Concentration of IAA Production by four PSB:

S. No	Isolates of PSB	IAA Concentration(mg/ml)
1	Isolate-A(Pseudomonas)	0.10
2	Isolate-B(Bacillus)	0.08
3	Isolate-C(Rhizobium)	0.15
4	Isolate-D(Azotobacter)	0.18

Result of Effect of PSB on mung bean plant after 10 days

Isolate	%Seed Germination	Root Length (cm)	Shoot Length (cm)	Vigour Index
Control	60	2.3	6.5	528
A	93	3.2	8	1023
B	86	3.0	7.5	903
C	73	3.6	7.5	810.3
D	80	3.6	6	336

Vigour index = % germination × seedling length (shoot length + root length)

Result of growth of isolates on their selective agar medium.

Result of total soil phosphate(before and after the plant growth):

Soil Sample	Orthophosphate (mg/gm)A	Acid hydrolysis polyphosphate $B=(A+B-A)(\text{mg/g})$		Acid digestion Organic phosphate	Total phosphate (mg/g)
		A+B	B	$C=D-A+B$	D
Initial	0.025	0.0347	0.0097	0.02125	0.06875
Final	0.01875	0.0203	0.0015	0.0172	0.0375

Result of soil parameters (before and after the plant growth):

Soil Sample	PH	Electrical Conductivity (mS/cm)	Moisture%	Organic Carbon(mg/g)
Initial	7.75	2.04	25%	7.87
Final	7.3	1.75	31.70%	8.62

Result of effect of PSB on mung bean plant:

Sr. no	Soil In Pot	Date of Sowing	Date of Germination	No. of Plant Germinated	No. of Leaves/ Plant	Leaves Length (cm)	Colour of Leaves	Plant Length (cm)
1	Control only soil	10/02/2020	10/03/2020	17	9	2.4	Green	17
2	Soil +isolate A	10/02/2020	10/03/2020	18	8	1.7	Green	18
3	Soil +isolate B	10/02/2020	10/03/2020	18	8	2.0	Green	18
4	Soil +isolate C	10/02/2020	10/03/2020	17	9	2.0	Green	17
5	Soil +isolate D	10/02/2020	10/03/2020	18	8	1.5	Green	19

4. CONCLUSIONS

The search for plant growth promoting phosphate solubilizing bacteria are isolated for used them as biofertilizer for various economically important crops. Isolates were harbours the plant growth promoting traits is the most promising properties to be developed as an inoculum for agro ecosystem. The present study was successful approach in selecting effective isolates from Rhizospheric soil sample that can be a useful component of plant growth promotion. All the isolates could be used as biofertilizer for mung bean plant.

Our finding indicate that most of the selected strains possess many plant growth promoting properties that significantly improve the growth parameters of the mung bean plants when tested under controlled condition. The use of this inoculum of phosphate solubilizing bacteria with own function is of interest as these formulations may increase consistency in the field. Phosphate solubilizing bacteria offer an environmentally sustainable approach to increase crop production and health.

In current study, four isolates were obtained successfully from Rhizospheric soil of tomato plant. Among four isolates, all four isolates are capable to solubilize insoluble phosphate from the soil and also produce plant growth promoter indole acetic acid (IAA). Among them two isolates, isolate-A (*Pseudomonas*) and isolate-B (*Bacillus*) were highly active phosphate solubilizers than isolate-C (*Rhizobium*) and isolate-D (*Azotobacter*). And from the four isolates, isolate-C (*Rhizobium*) and isolate-D (*Azotobacter*) are highly able for production of indole acetic acid (IAA) as compare to isolate-A (*Pseudomonas*) and isolate-B (*Bacillus*). Isolates A, B, C show good vigour index, except control and isolate D. These isolates were useful to survive in natural environment and help plant to grow in stressed environment condition. Isolate-A was found to be most effective in phosphate solubilizers and isolate-D was found to most effective in production of IAA. And these all are used as bio fertilizer.

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