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Review

Synergy between Plants and P-Solubilizing Microbes in soils: Effects on Growth and Physiology of Crops

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Phosphorous (P) is an essential macronutrient required by the plants for their vital functions such as photosynthesis, proteins and nucleic acid production, nitrogen fixation, formation of oil, sugars, starches *etc.* It is also the part of all biogeochemical cycles in plants. It is least mobile element which is available to plants as phosphate anion. P in precipitated form *i.e.* Orthophosphate ($H_2PO_4^{-1}$ or HPO_4^{2-}), is absorbed by Fe^{3+}, Ca^{2+} or Al^{3+}oxides in soil through legend exchange. A large amount of P applied as a fertilizer becomes immobile through precipitation reaction with highly reactive and Fe^{3+} in the acidic, and $^{+}$ in calcareous or normal soils. The use of phosphate solubilizing bacteria (PSB) as inoculants in soil increases the phosphorous uptake by the plants and also the crop yield. The ability of phosphate solubilizing bacteria to convert insoluble form of phosphorous into soluble one is an important trait in sustainable farming for increasing crops yield. PSB play an important role in enhancing phosphorous availability to plants by lowering soil pH and by microbial production of organic acids and mineralization of organic P by acid phosphatases. These organisms besides providing P also facilitate the growth of plants by improving the uptake of nutrients and stimulating the production of some phytohormones. PSB have high potential as bio-fertilizers especially in P-deficient soils to enhance the growth and yield performance of crops. The present article describes the progress of research on this area and future insights about use of PSB in agriculture.

Keywords: Plants, P-Solubilizing microbes, physiology of Crops

INTRODUCTION

The world population is increasing day by day (Lal, 2000), hence there is need for plenty of food crops to meet the requirement of growing population. Crops need several nutrients to reach their maximum potential yield. Most of the plants grow by absorbing nutrients from the soil and their ability to do this depends on the nature of the soil. Soil texture and its acidity determine the extent to which the nutrients are available to the plants. The nutrients, which are required by the plants, occur naturally in the soil but sometimes these are added as lime or fertilizer into the soil. Phosphorous is one of the essential elements required in optimum amounts for the growth and development of the plants (Bagyaraj *et al.*, 2000). About 98% soils have inadequate supply of available Phosphorous (Hansan, 1996) and likely to induce deficiency of this mineral.

Phosphorous (P) has several roles in the plants and is involved in functioning of nucleic acids, proteins, photosynthesis and in the formation of oils, sugars and starches *etc*. It is helpful in the rapid growth of the roots and shoots. Most of the soils contain the substantial reserves of total P; large part of it relatively remains inert and only less than 10% of soil P enters the plant-animal cycle (Kucey *et al.*, 1989). When P is added as fertilizer to the soil, it gets fixed. The soil microorganisms solubilise this P and make it available to the plants (Pal, 1998; Hilda and Fraga, 1999). P-solublising bacteria are relevant in this context and have the potential to be used as biofertilizer for the crops. We describe here their role in P solublisation in soil and consequent effects on agriculturally important plants with updated information.

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Phosphorous in Soil: Status and Availability

P is present in several hundred to several thousand grams of per acre in the soil, but its large amount in soils is not available to growing plants. Phosphate in the soil solution's P-pool is immediately available but the amount is very small in comparison to the total P in soils (Bushman *et al.*, 2009). In soil, the active P-pool is phosphorus that can be released into solution but is generally small in comparison to its fixed form. P forms the 0.12% of the earth crust. Knowing the fertility status of P in soil is helpful in determining the level of Phosphate fertilizer to be applied to crops. The amount of phosphorous which is present in the soil is 0.05%, out of this only 0.1% is available for the plants (Scheffer and Schachtshabel, 1988).

About 50% of the districts in India need higher levels of P in soils than are currently being used (Hasan, 1996). The reserves of P on earth include rocks and other deposits such as primary apatites and other primary minerals formed during the geological age (Fernandiz *et al.*, 1988). Apatite is the largest reservoir of phosphate on Earth (Stevenson, 1986) and is less soluble in water. Majority of P applied to soils is fixed rapidly and is not available to the plant roots. Especially, the soils of tropical areas are acidic in nature and are deficient in P. These soils have very low concentration of soluble P (Gaume, 2000; Goldstein, 1994).

P in the soil forms the orthophosphate ions complex with Ca, Al, Fe *etc.* (Khan *et al.*, 2009). It is present in the form of $H_2PO_4^-$ and HPO_4^- for the uptake by the plants that is known as the mineral phosphate solubilisation (MPS) (Bagyaraj *et al.*, 2000). The concentration of P in the soil is very low; it is about the level of 1 ppm or less (Rodriguez and Fraga, 1999).

P is one of the essential nutrients and is classified as macronutrient because it is required in large amounts by the plants (Bushman et al., 2009). P is one of the three nutrients which are generally added to soil in fertilizers. About 30 to 50% of the P in soil occurs in organic forms (Rodriguez and Fraga, 1999). The P fixation and precipitation is highly dependent on soil pH and type, thus, in acidic soils free oxides and hydroxides of Al and Fe fix P and in alkaline soils it is fixed by Ca, which causes its low efficiency (Goldstein, 1986).

Plant available nitrogen is present in millimolar amounts, while the plant available phosphorous is usually in micromolar amounts (Anthony et al., 2009). The organic phosphorous in the soil is largely in the form of inositol (soil phytate), synthesized by microorganisms and plants and is most stable (Anderson, 1980; Harley, 1983). The phosphorous in bound form is made available to the plants by soil microorganisms like bacteria and fungi, which solubilize the bound form of phosphorous and make it available to the plants (Jisha and Mathur, 2006).

The other common forms of organic phosphorous are phosphomonoesters, phosphodiesters including nucleic acids, phospholipids, glycerophosphate, sugar phosphate and coenzymes (Martinez *et al.*, 1968). These organic forms must be converted into inorganic phosphate or low molecular weight organic acids before they can be assimilated by plants (Figure.1). The organic forms are utilized by plants after mineralization and subsequent release of inorganic phosphorous (Yadav and Tarafdar, 2001). Plants complete their phosphorous requirement by uptake of phosphate anions from the soil solution (Richardson et al., 2000). Many of the phosphorous compounds have high molecular weight, therefore these must first be converted to either soluble phosphate (Pi, HPO_4^{-2}, $H_2PO_4^-$), or low molecular weight organic phosphate, to be assimilated by the plant cell (Goldstein, 1994).

P is the growth limiting nutrient and its biggest reserve is rock phosphate, which is highly insoluble. Agricultural soils have the large amount of organic and inorganic phosphorous, but this is unavailable for plant's use. This is due to the high reactivity of P with some metal complexes such as Fe, Al and Ca, leading to precipitation and adsorption of P in soil (Fig.1; Anthony *et al.*, 2009).

P is not found in elemental form because this form is extremely reactive. It combines with oxygen when exposed to air. In natural system like soil and water, P exists as phosphate, a chemical form in which phosphorous is surrounded by oxygen atoms (Hyland et al., 2005). Orthophosphate is the simplest phosphate with chemical formula PO_4^{-3}. In water, orthophosphate mostly exists as $H_2PO_4^-$ in acidic condition or as HPO_4^{-2} in alkaline condition (Bushman *et al.*, 2009).

Role of Phosphorous in Plants

P is the most important nutrient required by the plants for growth and development. It is the second major essential macronutrient and plays an important role in metabolism of crop plants (Vikram and Hamzehzarghani, 2008). About 10-25% of fertilizer P is acquired by the plants (Saha and Biswas, 2009) for promoting their functions. For instance, PSB have been found to promote the nitrogen fixation, yield and nutrient uptake in chickpea (Saber *et al.*, 2005; Wani *et al.*, 2007).

P is absorbed mainly during the vegetative growth; therefore most of its absorbed form is re-translocated in fruits and seeds during the reproductive stages. Phosphorous is the important nutrient for plant growth (Eftkhari *et al.*, 2010) and it is the constituent of various cellular functions or activities such as cell division, development, photosynthesis, breakdown of sugars, nutrient uptake and transport within the plant (Griffith, 1999). The plants which are deficient in P, show retarded growth and causes dark green colouration due to enhancement of anthocyanin formation (Khan et al., 2009).

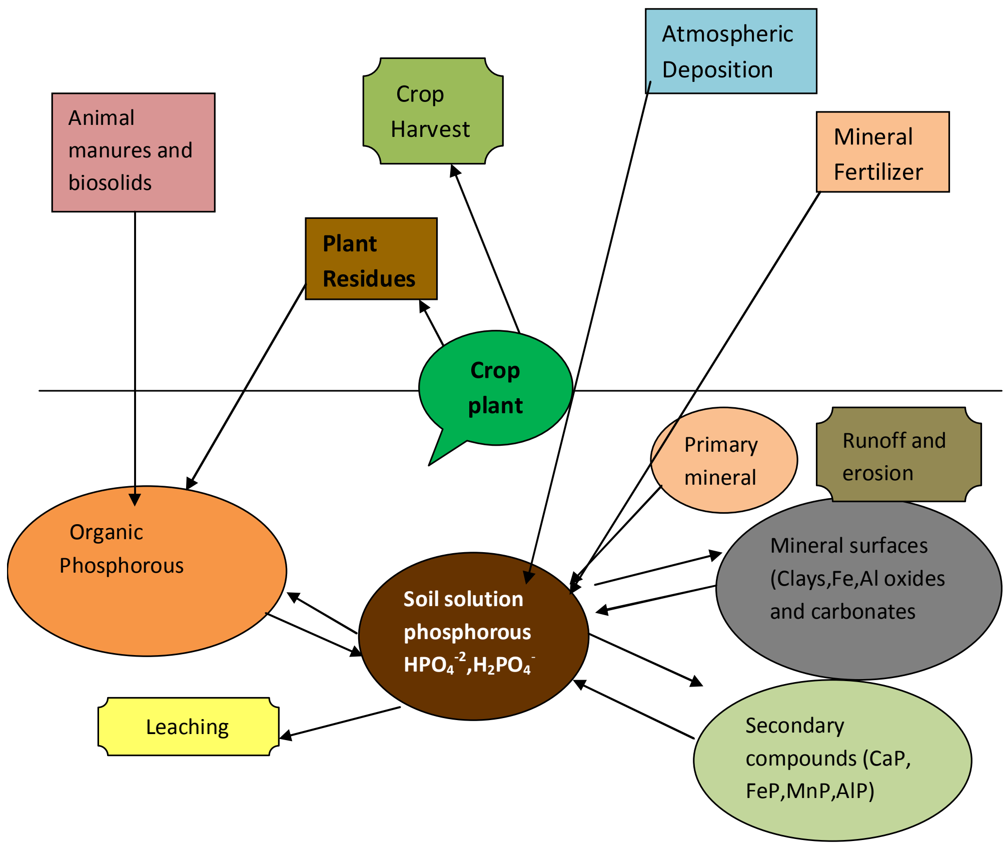

Figure 1. Phosphorous Cycle in nature

Phosphate solubilising Bacteria (PSB)

Soil is a dynamic system and is an ecological niche of constant biological activity (Bagyaraj *et al*., 2000). Soil bacteria play an important role in biogeochemical cycles. These have been used for decades in crop production (Hayat *et al*., 2010). There are number of bacterial species, which have the capability to change the insoluble form of phosphorous into soluble one and they are known as the phosphate solubilising bacteria (PSBs). They are also known as the plant growth promoting rhizobacteria (PGPR) because they colonize the plant roots and promote growth to the plants. There are the two levels of complexity in relationship between plant growth promoting rhizobacteria and host plant. These levels are rhizopheric and endophytic (Hayat *et al*., 2010).

Phosphate solubilising bacteria (PSB) have been used for the crop production since 1903. These bacteria play an important role in supplying phosphate to plants, in environment friendly and sustainable manner (Khan *et al*., 2007). Plant roots provide food, shelter, energy and the areas of very high biological diversity and these areas are known as 'rhizospheres' (Mc Millan, 2007). PSB are capable of solubilising accumulated phosphatic compound sources in soil by production of organic acids, phenolic compounds, protons and siderophores (Landweet *et al*., 2001).

Phosphate solubilising microbial communities differ in their structure and functionalities on the basis of phosphorous present in the soil; therefore response of these communities towards this component is important for formulating management strategies (Saha and Biswas, 2009). PSB promote the plant growth, reduce diseases or insect damages. Phosphate solubilising microorganisms constitute 1 to 50% in phosphorous solubilisation potential (Chen *et al*., 2006). Introduction of genes, which are involved in soil phosphate solubilisation in natural rhizosphere is a very useful approach for improving the ability of PSB to be used as inoculants (Bashan *et al*., 2000). The insertion of these genes into the bacterial chromosomes is advantageous for stability and ecological safety (Rodriguez *et al*., 2006).

Since agricultural soils have the inadequate supply of P; therefore application of phosphate fertilizers is must to complete the requirement of P in soil (Vikram and Hamzehzarghani, 1998). PSB solubilize this P and make it available to the plants. Recently, PSB have also been used in soil for the mineralization of pollutants i.e. bioremediation of polluted soil (Middlerop *et al*., 1990; Burd *et al*., 2000).

Table 1. *Bacillus sp.*

Bacteria	Reference
B.mycoides, B.polymyxa	Gaind and Gaur, 1990; Kaul *et al*., 1999
B.licheniformis, *B.amyloliquefaciens, B.atrophaeus*	Vazquez, 2000
B. megaterium, B. phosphaticum	Sundara *et al*., 2002
B. cerus	Chandra *et al*., 2007
B. subtilis	Chatli *et al*., 2008
Paenibacillus marceras	Krishnaewamy *et al*., 2009

Table 2. *Pseudomonas sp.*

Bacteria	Reference
P. syringae, P. aeruginosa	Bardiya and Gaur, 1974
P. flouresence	Nautiyal, 1999
P. striata	Peix *et al*., 2004

Types of PSB

There are number of bacterial species, which are being used worldwide with the aim of enhancing plant productivity (Burd *et al*., 1998; Cocking, 2003). Bacteria that are used may be symbiotic or non- symbiotic. Symbiotic bacteria include *Rhizobium* sp. and non-symbiotic include *Azotobacter*, *Azospirillum*, *Bacillus* and *Klebsiella* sp. etc. (Khan, 2005).

PSB include *Pseudomans, Bacillus, Enterobacter, Azospirillum and Rhizobium* etc. and are also called as rhizobacteria because they colonize the plant roots and promote plant growth (Antoun and Kloepper, 2001). The free living bacteria, which are beneficial to crops are termed as the plant growth promoting rhizobacteria (PGPR); they are capable of enhancing plant growth by colonizing the roots of the plants (Kloepper and Schroth, 1978). Plant growth promoting rhizobacteria or nodule promoting bacteria (NPR) are associated with the rhizosphere of the soil that is an important ecological environment for the plant-microbe interaction (Burr and Caesar, 1984).

On the basis of relationship with the plants, PGPR are divided into two groups: Symbiotic and free living bacteria (Khan, 2005). On the basis of their living sites PGPR have two groups:

- iPGPR
- ePGPR

iPGPR (i.e., symbiotic bacteria) are the group of bacteria which live in specialized structures inside the plant cell, on other hand, ePGPR (i.e., free living bacteria) are the group of bacteria which live outside the plant cell (Gray and Smith, 2005). iPGPR have the capability to produce the nodules inside the cell, on other hand ePGPR do not produce nodules but they still enhance the plant growth. Rhizobium is the best known iPGPR which produce nodules in leguminous plants.

The paramount and most efficient PSB belong to genera *Bacillus* and *Pseudomonas*. *Bacillus* and *Paenibacillus* are specifically used to enhance the status P in plants (Brown, 1974). *Rhizobium sp.* such as *Rhizobium, Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium and Sinorhizobium* in combined inoculation with phosphate solubilising bacteria, are used to enhance the plant productivity (Akhtar and Siddiqui, 2009). Some of the *Bacillus and Pseudomonas* species which are used as Phosphate solubilizers are given in table 1 and table 2.

There are some non-phosphate solubilising bacteria, which are also important as P biofertilizers. These bacteria can take up soluble phosphate from the soil through their affinity transporters and make it available to the plants through mineralization, as the bacteria die (Gyaneshwar *et al*., 2002). A list of the phosphate solubilising bacteria is given in table 3.

Some of the groups of fungi are also used to enhance the crop productivity and to solubilize rock phosphate. These mainly include the various species of genera *Aspergillus* and *Penicillium* (Kang *et al*., 2002) (Table 4).

Occurrence and mode of action of PSB

Microorganisms are ubiquitous and usually, one gram of fertile soil contains 10^1 to 10^{10} bacteria, and their life may exceed 2,000 kg ha^{-1} (Hayat et al. 2010). Soil bacteria are found in various forms, they may be cocci, bacilli or spiral. Bacilli are common in soil and are mostly used in phosphate solubilisation in soil, but spirilli are very rare in natural environment (Baudoin *et al*., 2002).

There is the great variation in form and population of PSB in soil. Population of PSB in soil depends upon its chemical and physical properties and also on organic matter and phosphorous content of soil (Kim *et al*., 1998).

Table 3. Phosphate Solubilising Bacteria

Bacteria	Reference
Xanthomonas sp., Flavobacterium	Swaby and Sperber, 1958.
Pseudomonas sp., Pseudomonas striata	Vidyasekaran, 1973.
Rhizobium japonicum, Rhizobium leguminosarum, Rhizobium sp.	Reichlova, 1972; Gostkowska, 1976, Shingte *et al.*, 1987;
Azotobacter chroococcum	Kundu and Gaur, 1980.
Enterobacter aerogenes, Enterobacter agglomerans.	Thakkar *et al.*, 1993, Kim *et al.*, 1997
Burkholderia cepacia	Maheshkumar., 1997.
Acetobacter sp.	Santhi, 1998.
Azotobacter sp.	Zahir *et al.*, 2000.
Kluyvera ascorbata	Burd *et al.*, 2000.
Azospirillum brasilense, Azospirillum lipoferum	Thakuria *et al.*, 2004; Muratva *et al.*, 2005.
Mycobacterium sp.	Beneduzi *et al.*, 2007.
Bacillus cerus, Bacillus megaterium, Bacillus subtilis	Chandra *et al.*, 2007; Chatli *et al.*, 2008.
Streptomyces acidiscabies	Dimpka *et al.*, 2008.
Acinetobacter calcoaceticus	Peix *et al.*, 2009.

Table 4. Fungal groups

Fungi	Reference
Aspergillus tubingensis, Aspergillus niger	Richa *et al.*, 2007.
Penicillium expansum, Penicillium sp.	Kucey *et al.*, 1987; Cunningham *et al.*, 1992; Fenice *et al.*, 2000; Mittal *et al.*, 2008.
Trichoderma sp.	Altomere *et al.*, 1999; Ahmed, 2010.

PSB are concentrated in the rhizosphere since this is the metabolically most active region (Vazquez, 2000). Rhizosphere is the zone surrounding the roots of plants in which the complex relations exist among the plants, soil microorganisms and the soil itself (Krishenvani, 2010).

PSB belong to diverse taxonomic groups of bacteria and the ecological role of these bacteria in soil is very important, because they take part in biogeochemical cycles of phosphorus in the ecosystems, Therefore, it is necessary to study the composition and dynamics of these bacterial populations to reach a better understanding of soil microbial diversity, nutrient transformation and uptake by plants (Saha and Biswas, 2009). Mode of action of these PSB include increasing the surface area of the plant roots, increasing the availability of the nutrients in the soil to the plants, assisting the nitrogen fixation and enhancing the other beneficial effects of symbiosis on the host: (Figure 2.)

Mechanism of Solubilisation

There are various mechanisms by which microorganisms solubilize inorganic phosphate. It can be by secretion of organic acids (Goldstein, 1995) or by production of siderophores (Vassilev et al., 2006). The secretion of phenolic compounds and humic substances is also reported (Patel *et al.*, 2008).

PSB solubilize phosphate by production of organic acids. There are various heterotrophic microorganisms, which help in excretion of organic acids, they dissolve phosphatic minerals or chelate cationic partners of the phosphate ions i.e. PO_4^{-3} and directly release phosphorous into the soil (Khan *et al.*, 2009). In soil, these organic acids reduce the pH of their surroundings (Goldstein, 1994). These acids can either dissolve the phosphorous directly by lowering the pH of soil, which can help in ion exchange of PO_4^{2-} by acid ions or they can chelate heavy metal ions such as Ca, Al and Fe and release associated phosphorous with them (Bardiya and Gaur, 1972; Moghimi *et al.*, 1978).

The two reactions, fixation and immobilization convert the applied phosphorous into the forms, unavailable for the plants (Bagyaraj and Varma, 1995). Immobilization occurs, when the phosphorous, which is plant available, is consumed by microbes, turning phosphorous into organic forms that are not available to plants. General sketch of P solubilisation in soil is shown in Figure 3. This P becomes available over time as microbes die (Hyland

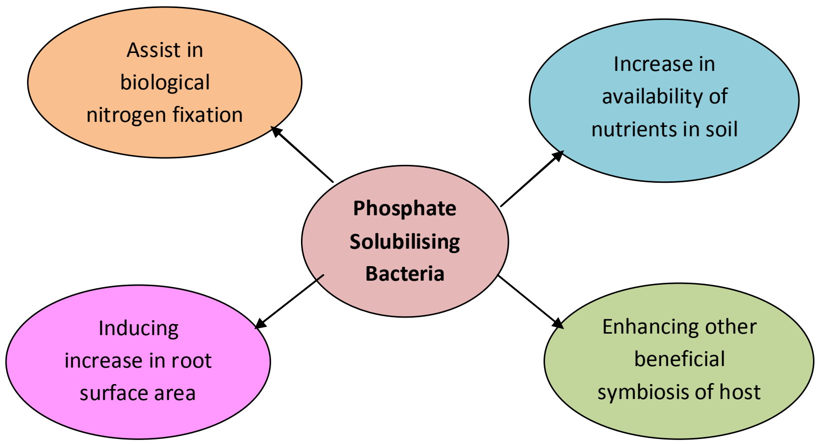

Figure 2. Mode of Action of PSB

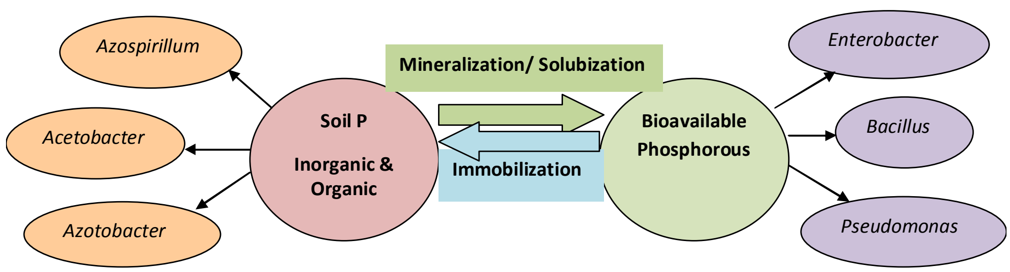

Figure 3. Soil Phosphorous mobilization and immobilization by bacteria

et al., 2005).

Mineralization is the microbial conversion of organic phosphorous, which is available in the soil, to $H_2PO_4^-$ or HPO_4^{-2}, forms of plant available phosphorous, known as orthophosphates. The ability of the microbes to solubilize P depend upon their ability to produce organic acids, which through their hydroxyl and carboxlic groups chelate the cations bound to phosphate and then make it available for the plant use (Sagoe *et al.*, 1998). Microbes solubilize phosphorous by organic acid production and proton extrusion (Nahas, 1996; Dutton and Evans, 1996).

Soil microbes dissolve the soil P by production of low molecular weight organic acids (Goldstein, 1995). Organic acids, which solubilize phosphorous are mainly citric acid, lactic acid, gluconic acid, 2-ketogluconic acid, oxalic acid, tartaric acid and acetic acid *etc.* (Ivanova *et al.*, 2006). Out of these, gluconic acid and ketogluconic acid are mainly produced by soil microorganisms (Goldstein.1995). These organic acids are the source of biotical generated H^+ ions, which are able to dissolve the mineral phosphate and to make it available for the plants (Bhattacharya and Jain, 2000). PSB also produce auxins such as indole-3-acetic acid (IAA) and indole-3-ethanol chrom-azurol as plant growth regulators (Egamberdiyeva, 2005; Chandra *et al.*, 2005; Roesti *et al.*, 2006; Dell'Amico *et al.*, 2008;). The mechanism of phosphorous solubilisation involves:

- lowering of pH by biotic production of proton/bicarbonate release
- gaseous exchange
- chelation of cations
- and by competing with phosphorous for the adsorption sites in the soil (Nahas, 1996).

Some of the inorganic acids (e.g. HCl) are also helpful in solubilizing phosphorous, but they are less effective as compared to organic acids (Kim *et al.*, 1997).

Effects of PSB on Plants

PSB have the various beneficial effects on the plants. These bacteria exert the direct or indirect effects on the plants. Direct effects include the increased solubilisation and uptake of nutrients or production of plant growth regulators while the indirect effects include suppression of pathogens and producing metal binding molecules, known as siderophores (Hayat *et al.*, 2010). PSB enhance the plant growth and yield because:

- they have ability to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase to reduce the level of ethylene in roots thereby increasing root length and growth (Penrose and Glick, 2001).

- they enhance the biological nitrogen fixation in plants (Kennedy *et al*., 2004).
- they produce metal binding molecules siderophores (Pal et al., 2001), β-1,3 glucanase, fluorescent pigments, chitinases, antibiotics and cyanides to protect plants against pathogens (Cattelan *et al*., 1999).
- they have ability to produce different types of hormones like auxins, abscisic acid (ABA), gibberellic acid and cytokinins (Dey *et al*., 2004).
- they provide resistance to drought, salinity, water-logging and oxidative stress (Alvarej et al., 1996; Saleem et al., 2007; Stajner *et al*., 1997) and help in the solubilisation and mineralization of nutrients (Richardson, 2001).
- they produce water soluble vitamins like niacin, thiamine, riboflavin and biotin for plant growth (Revillas *et al*., 2000; Sierra *et al*., 1999).
- they synthesize specific compounds like hormones, enzymes (e.g. 1-aminocyclopropane-1-carboxylate) *etc*. which are required for the plant growth (Dobbelaere *et al*., 2003).
- they promote free living nitrogen fixing bacteria and enhance nitrogen fixation and the supply of nutrients like phosphorous, sulphur, iron and copper (McMillan, 2007; Cakmakci *et al.,* 2006).
- they prevent the crop plants from pathogens and diseases (Guo *et al*., 2004; Saravana *et al*., 2008).

Application on Crops

Most of the bacterial species isolated from the soil have the ability to dissolve the rock phosphate both in soil and culture medium, by secreting low molecular weight organic acids, which attack the phosphorus structure and make it available to the plants (Ivanova *et al*., 2006). Phosphorous compounds, which are insoluble in soil are solubilized by organic acids, phosphatase enzymes and complexing agents produced by plants and microorganisms (Park *et al*., 2009). PSB play an important role in enhancement of growth and yield of crop plants by providing them phosphorous, which is otherwise unavailable to plants (Gyaneshwar et al., 2002).

The effective strains of PSB are used to increase the level of P in the soil. With increase in the level of P, there is overall increase in the plant growth. Symbiotic relationship was observed between the PSB and crop plants, as soluble phosphorous was provided by bacteria for the plants that in turn provide carbon (Rodriguez and Fraga, 1999). These bacteria are useful in enhancement of yield performance cereals, legumes, oil seed crops, horticultural and fibre crops, Here, we present some recent observations about the effects of PSB on different types of crops.

❖ Leguminous crops

In gram (*Cicer arietinum*), the yield response and nutrient uptake was increased following seed inoculation with *Rhizobium* and PSB namely *Pseudomonas striata* and *Bacillus polymyxa* under field conditions. Increase in nodulation, nitrogenase activity, dry matter content was observed that was associated with significant increase in uptake of nitrogen and phosphorous over uninoculated strains (Alagawadi and Gaur, 1988). In another study on gram, Tomar *et al*. (1996) tested the efficiency of a PSB (*Pseudomonas sp.*) on the growth and yield of gram (*Cicer arietinum*) that resulted in increase in its growth and grain yield. Among the sources of P, rock phosphate and pyrite proved to be best to enhance the grain yield.

In soybean, the application of PSB *Pseudomonas sp.* enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (*Glycine max*) (Son *et al.,* 2006).

In green gram (*Vigna radiata*), the inoculation with different PSB isolates like *Pseudomonas, Bacillus, Xanthomonas, Serratia* and *Enterobacter* resulted in higher nodule number, nodule dry weight, shoot dry matter and total dry matter. Majority of PSB were able to improve growth parameter of green gram significantly compared to rock phosphate control and single superphosphate control (Vikram and Hamzehzarghani, 2008).

Seed inoculation of cowpea (*Vigna unguiculata*) by *Gluconacetobacter sp.* and *Burkholderia sp.* helped in improved nodulation, root and shoot biomass, straw and grain yield and phosphorous and nitrogen uptake of crops. Out of these, best results were shown by *Burkholderia sp.* (Linu *et al*., 2009).

❖ Cereals

In pot experiments conducted to study the effect of inoculation with pure and mixed cultures of nitrogen fixers *Azospirillum lipoferum, Arthobacter mysorens* and PSB strain *Agrobacterium radiobacter* on growth and mineral nutrition of two barley cultivars, a positive effect on grain yield, nitrogenous nutrition and growth of both the barley cultivars was obtained after inoculation with mixtures of these strains (Belimov *et al*., 1995).

Twenty seven PSB including seventeen bacteria and ten fungal isolates were isolated from the rhizosphere soil of crop plants. Out of these, *Aspergillus niger* and *Penicillium vermiculosum* were found to be the most efficient strains and the four bacteria *Bacillus sp.* and *Pseudomonas stutzeri* were selected to test their ability to solubilize phosphates in liquid media. These were tested on wheat which showed great yield and nutrient (*Triticum* *aestivum*) (Jisha and Mathur, 2005).

Gram positive PSB *Bacillus* showed significant effects on winter wheat, total phosphorous and plant biomass both under pot and field conditions. It was observed that these bacteria had the capability to convert the non-available forms of phosphorous into plant available forms (Chen *et al*., 2006).

Inoculation of maize (*Zea mays*) with two efficient screened strains i.e. *Serratia marcescens* and *Pseudomonas sp*., both under greenhouse and field conditions, showed the increased plant biomass. These both strains survived upto 96 days after sowing (Hameeda *et al*., 2006).

In order to study the effect of phosphate solubilisation by PSB (*Azotobacter coroocoocum, Azospirillum brasilens*, *Pseudomonas putida, and Bacillus lentus*) on yield and growth components of corn (*Zea mays*), an experiment was conducted. Increased row number, ear weight, grain number/year, grain yield, biological yield and harvest index was observed as compared to control (Yazdani *et al*., 2009).

❖ Oil seed and fibre crops

A field experiment was conducted for three years to evaluate the performance of groundnut (*Arachis hypogea*) under alluvial soils of eastern India with different types of inoculants such as *Rhizobium*, PSB (*Bacillius polymyxa*), no inoculants and different levels of cobalt. Higher yield and nutrient uptake was observed with inoculation of *Rhizobium* and *Bacillus polymyxa*. Also, the kernel yield was recorded to be highest, which was 16.50% higher over no inoculants respectively (Basu and Bhadoria, 2008).

Ekin (2010) investigated the efficiency of PSB, *Bacillus* M-13 on the growth and productivity of sunflower (*Helianthus annuus*) which resulted in the improved seed quality and oil yield. An increase in head diameter, 1,000 seed weight, kernel ratio and oil content was observed which led to the seed and oil yield increase of 15 and 24.7% over no application, respectively.

Stimulatory effects of bacterial species such as *Pseudomonas*, *Bacillus*, *Arthrobacter* and *Rhizobium* was observed on growth, yield, nitrogen and phosphorous uptake of cotton (*Gossipium Hirusitum*). The results revealed the increase in root and shoot length and also the soil phosphorous content (Egamberdiyeva *et al*., 2005).

A field experiment was conducted on cotton crop with *Bacillus sp*. Results showed that *Bacillus sp.* significantly increased the seed cotton yield, number of bolls/plant, boll weight, plant height, staple length, plant phosphorous and available phosphorous in the soil (Akhtar *et al*., 2010). It was concluded that PSB not only exert beneficial effects on crop, but also enhance the phosphate concentration in the soil.

❖ Vegetable and horticultural crops

Biofertilizers have been used as sources to improve plant nutrients in sustainable agriculture. To evaluate the potential of PSB *Bacillus megaterium* var. *phosphaticum* and potassium solubilizing bacteria (KSB) *Bacillus mucilaginosus*, an experiment was conducted in nutrient limited soil planted with pepper and cucumber. Results showed that rock P and K applied either singly or in dual combination did not significantly enhance soil availability of P and K, indicating their unsuitability for direct application. PSB was a more potent P-solubilizer than KSB, and co-inoculation of PSB and KSB resulted in consistently higher P and K availability than in the control without bacterial inoculum and without rock material fertilizer. Integrated rock P with inoculation of PSB increased the availability of P and K in soil, the uptake of N, P and K by shoot and root, and the growth of pepper and cucumber (Han *et al*., 2006).

In another experiment, the effects of PSB (*Bacillus* FS-3) application were studied on phosphorous content of tomato (*Lycopersicon esculentum*) under green house conditions with five different fertilizer treatments. A greater increase was noticed in plant root and shoot weight and phosphorous uptake in treatments with PSB application than without PSB in all of fertilizer treatments (Turan *et al.,* 2007).

Tomato (*Lycopersicon esculentum*) is one of the important vegetable crops. Tantawy *et al*. (2009) observed the effects of inoculation with phosphate solubilizing bacteria on tomato rhizosphere, colonization process plant growth and yield. The inoculation with *Pseudomonas sp.* and manuring with different organic sources had a positive significant effect on tomato leaves phosphorous content.

Phosphorous is the key nutrient required by the sugarcane (*Sacchrum officinarum)* for the higher productivity of sugar. Application of phosphate solubilizing bacteria, *Bacillus megaterium var. Phosphaticum,* with varying amounts of phosphorous fertilizer, on sugarcane increased the sugarcane growth and yield and the status of available phosphorous in the soil. Enhanced tillering, stalk population and stalk weight was observed, which led to increase in cane yield (Sundara *et al*., 2002).

Enhancement in the biosynthesis of furanone which is the flavour compound in strawberry (Fragaria x ananassa) was observed on inoculation with PSB *Bacillus subtilis* (Zahetakis, 1997). Also, inoculation of strawberry (Fragaria × ananassa) with different PSB such as *Bacillus subtilis*, *Pseudomonas fluorescens*, provided the control against diseases like Crown rot caused by *Phytophothora cactorum* and red steel caused by *Phytophothora fragari* (Vestburg *et al.,* 2004).

Furthermore, there are several examples of crop plants and their growth promotion and increase in phosphorous uptake by phosphate solublising bacteria which have

Table 5. Agronomic response of phosphate solublising bacteria

Bacteria	Crop	Response	Reference
Azotobacter and PSM	*Arachis hypogea*	Increased yield	Kundu and Gaur, 1980
Bacillus subtilis along with Glomus intraradices (AM fungi)	*Allium cepa*	Improved P bioavailability and nutrient cycling	Toro *et al.*, 1997
Bacillus sp.	*Amaranthus hypochondriacus, Phaseolus vulgaris, Fagopyrium esculentum*	Enhanced growth and yield and nutrient uptake	Pal, 1998
Glomus sp. *Bacillus circulans* *Cladosporium herbarum*	*Triticum aestivum*	Improved growth and yield	Singh and Kapoor,1999
Azotobacter sp.	Maize	Efficient IAA production and growth promoting effects	Zahir *et al.*, 2000
Kluyvera ascorbata	Canola and Tomato	Decreased plant growth inhibition by heavy metals (Ni, Pb, Zn)	Burd *et al.*, 2000
Bacillus amyloliquifaciens *Bacillus subtilis*	Tomato	Control against tomato mottle virus disease	Murphy *et al.*, 2000
Pseudomonas aeruginosa *Bacillus subtilis*	Mung bean	Prevent root rot and root knot disease	Siddiqui *et al.,* 2001
Rhizobacteria	Wheat and Rice	Increased yield, nutrient uptake and IAA production	Khalid *et al.,* 2001
Bacillus sp.	Cucumber	Growth and control against pathogens	Stout *et al.*, 2002
Bacillus subtilis *Aspergilus awamori* *Aspergillus niger* *Pseudomonas fluorescens*	*Solanum lycopersicum*	increased nutrient uptake and P bioavailability	Khan *et al.*, 2002
Strepyomyces marcescens *Baciliius pumilis*	Tobacco	Bio-control against blue mould	Zhang *et al.*, 2003
Azotobacter sp. Pseudomonas sp.	Wheat	Fungal bio-control	Wachowaska, 2004
Bacillus cereus MJ-1	Red Pepper	Increased P uptake, disease control and Increased plant biomass	Joo *et al.*, 2005
Pseudomonas sp. *Bacillus sp.* *Aspergillus sp.*	*Gossypium sp.*	Increased P uptake	Narula *et al.*, 2005
Enterobacterium	*Pisum sativum* and *Cicer arietinum*	Increased P uptake and biomass	Hynes *et al.*, 2008
Azospirillum brasilense	*Prunus cerasifera*	control against pathogens and increased yield	Russo *et al.*, 2008
Pseudomonas sp.	*Triticum aestivum*	Improved grain yield, shoot weight and plant height	Afzal and Bano, 2008
Thiobacillus	*Brassica napus*	Increased yield	Salimpour *et al.,* 2010

been tabulated as Above (Table 5):

Effects of Phosphate solublising bacteria on Physiology of Crop Plants

Phosphorous solublising bacteria aggressively colonize plant roots and induce plant growth and promotion (Saharan and Nehra, 2011). In most bacteria, mineral phosphate dissolving capacity has been shown to be due the production of organic acids (Rodriguez and Fraga, 1999). These bacteria can directly or indirectly affect the plant growth (Mantelin and Touraine, 2004). They act as the chemical messengers by producing hormones, which are effective at very low concentration. They are synthesized in one part of the plant and are transported to another location and affect a plant's ability to respond to its environment (Sahran, 2011).

Phosphate solublising bacteria assist in good supply of nutrients to plants, improve soil structure and also help in the bioaccumulation or microbial leaching of inorganic compounds (Brierley, 1985; Ehrlich, 1990). The plant-microbe interactions by PSB such as *Azotobacter, Azospirillum, Bacillus, Klebsiella, Pseudomonas etc.* in the rhizosphere play a vital role in transformation pathways, mobilization of nutrients and solublisation processes of nutrients from limited nutrient pool and subsequently uptake of essential nutrients by plants to realize their genetic potential (Hayat *et al*., 2010).

Plant growth promoting rhizobacteria (PGPR) produce and increase the synthesis of plant growth regulators namely, auxins, gibberellins, cytokinins, ABA and ethylene (Zahir *et al*., 2004). These phyto-hormones play a vital regulatory role in plant growth and development. PGPR influence other physiological processes of the plants through these hormones (Dobbelaere *et al*., 2003). Out of these hormones, auxin is the predominant and most active that is known to stimulate both rapid (e.g. increase in cell elongation) and long term (e.g. cell division and differentiation) responses in plants (Hagen, 1999; Cleland, 1990).

About 80% of the bacteria that are isolated from the rhizosphere are known to produce indole-3-acetic acid (IAA). Also, the bacteria like *Paenibacillus polymyxa* and *Azospirilla* release the regulators like Indole-3-butyric acid (IBA), tryptophan and tryptophol or indole-3-ethanol that can indirectly contribute to plant growth (Lebuhn *et al*., 1997). In same way, as many as 90% of microbes found in rhizosphere are capable of producing cytokinins when these are cultured *in vitro* (Barea *et al.,* 1976). The effect of cytokinins producing bacteria, *Azotobacter chrococcum*, was studied on the growth and morphology of radish and maize under greenhouse and field conditions. A significant improvement in plant growth was observed with application of these bacteria to these plants (Nieto and Frankenberger, 1991).

A number of bacteria such as *Azospirillum, Azotobacter* are known to produce gibberellic acid which is primarily responsible for stem elongation (Dobbelaere et al., 2003). In addition, abscisic acid has also been detected in supernatants of *Azospirillum* and *Rhizobium* sp. cultures (Dangar and Baso, 1987), which is an important component in stomatal movements and uptake and transport in plants. It is important for the plant growth under water stressed environment, such as is found in arid and semi-arid climates (Frakenberger and Arshad, 1995). Ethylene is potent plant growth regulator that affects many aspects of plant growth, development and senescence (Reid, 1987). The bacteria act as the sink for 1-aminocyclopropane-1-carboxylate (ACC). The hydrolysis products of ACC, ammonia and α-ketobutyrate are used by the bacterium as a source of nitrogen and carbon for the growth of plant (Klee *et al.,* 1991). Also, they lower the ethylene level in plants, preventing some deleterious consequences of high ethylene concentration (Steenhooudt and Vanderleyden, 2000; Saleem *et al.,* 2007).

Phosphate solubilizing bacteria such as *Bacillus megaterium* and *Pseudomonas sp*. enhance the ability of plants to fix atmospheric nitrogen and make it available to the plants (Chairarn *et al*., 2008). Among the soil bacterial communities, *Pseudomonas striata* and *Bacillus sircalmous* are known to improve the photosynthetic rate in plants. In salinity stress, due to decreased water uptake, plants exhibit reduced leaf growth, which restricts the photosynthetic capacity of the plants, but the inoculation of salt-stressed plants with these bacteria alleviates the salinity stress in plants (Hu C, 2005).

Gene Manipulation of PSB and Effects

Phosphorous plays an important role in plant energy transfer system. Deficiency of phosphorous causes growth retardation and tillering. Phosphorous is abundant in soil, but it reacts readily with elements i.e. Fe, Al and Ca to form insoluble compounds. These reactions result in very low phosphorous availability and low efficiency of phosphate fertilizers used by the plants (Jodie and Peters, 2000).

Bacteria have an elaborate system, which mineralizes the organic phosphate into Pi via enzyme alkaline and acid phosphates (Bagyaraj *et al*., 2000). Heterologous expression of genes in agriculturally important bacterial strains is necessary for improving organic phosphate mineralization in plant growth promoting rhizobacteria (Fraga *et al*., 2001). Insertion of transferred genes into the bacterial chromosomes is beneficial for safety and ecological safety (Hayat *et al*., 2010).

Manipulation of the genes of phosphate solublising bacteria is the another way to enhance the ability of phosphate solublising bacteria for the growth improvement of the plants (Rodriguez and Fraga, 1999). It improves the efficiency of the bacteria to solublising phosphorous and make it available for the plants. Acidification of the medium by biosynthesis and release of wide variety of organic acids is the most common way to solubilize tri- Ca phosphates (Igualet *et al.,* 2001; Delvasto *et al*., 2008). The genes from the gram negative bacteria, *Erwinia herbicola,* were first cloned by Goldstein and Liu (1987) for mineral phosphate solubilisation in *E.coli* that allowed the production of gluconic acid (GA) which had the ability to solublising hydroxyl-apatite. Another type of mineral phosphate solubilizing gene (gabY) involved in mineral phosphate solubilisation, was cloned in *E.coli* that induced the production of gluconic acid (Babu-Khan *et al.,* 1995).

E.coli does not produce gluconic acid because it is not capable of synthesizing apo-glucose dehydrogenase enzyme (GDH) and the cofactor pyrroloquinoline quinone (pqq). Some of the experiments showed that the expression of *Erwinia herbicola* gene in *E.coli* resulted in the production of gluconic acid (Liu *et al.,* 1992). The nucleotide sequence analysis of 7.0 kb fragment from the genomic DNA of *Rahnella aquatilis*, which is a gram negative bacterium, induced the mineral phosphate solubilisation on insertion into the *E.coli*. It showed the ability to solubilize hydroxyapatite and the production of gluconic acid in *E.coli.* It was observed that the amount of soluble phosphate gluconic acid produced by transgenic *E.coli,* was higher than those of *Rahnella aquatilis* (Kim *et al*., 1998). In another experiment, transgenic *E.coli* showed mineral phosphate solubilisation without changing the pH of the medium (Kim *et al.,* 1997). A cloned DNA fragment taken from the *Serratia marescens* and transferred into *E.coli* DH5α, was capable of producing gluconic acid and phosphate solubilisation. Gluconic acid production was regulated by this gene under cell-signal effects (Krishanraj and Goldstein, 2001). In another experiment, a phosphoenol pyruvate carboxylase (*ppc*) gene from *Synechococcus* appeared to be involved in mineral phosphate solubilisation. This gene showed an increase in *ppc* gene activity and resulted in increased carbon flow and enhanced production of gluconic acid by the direct oxidation pathways in the presence of soluble phosphate (Buch *et al.,* 2008).

Plants are not able to obtain phosphorous directly from the phytate, the primary source of inositol and major stored form of phosphate in plants. The growth and phosphorous nutrition of *Arabidopsis* plants was improved significantly, when they were genetically transformed with phytase gene isolated from *E.coli* (Golovan *et al*., 2000). Moreover, an increase in extracellular phosphatase activity of recombinant bacterial strain was also achieved. These all observations demonstrate the complex mechanisms of mineral phosphate solubilisation in different bacterial strains and give the basis for the understanding of process.

Association of PSB with other microorganisms

❖ PSB and *Rhizobium*

Symbiotic nitrogen fixers and PSB play an important role in supplementing the nitrogen and phosphorous to the plants and thereby allowing a sustainable use of nitrogen and phosphate fertilizers (Tambekar *et al*., 2009). *Rhizobium* is a symbiotic nitrogen fixer and it was observed in field experiments that depending upon the legume, soil and climatic conditions about 50% of the nitrogenous fertilizers could be saved through inoculation of *Rhizobium* in combination with PSB like *Bacillus, Azospirillum, Pseudomonas* etc (Rewari *et al.,* 1972; Tilak, 2005).

Rhizobium in combination with PSB increases phosphorous nutrition by the mobilization of the organic and inorganic phosphate (Alikhani *et al*., 2006). In chickpea, an increase in nodulation, growth, and nutrient content and yield parameters was observed with combined inoculation of *Rhizobium* and PSB (*Pseudomonas striata* and *Bacillus polymyxa*) under greenhouse conditions. This was associated with increase in nitrogenase activity in nodules and phosphorous content in plants (Algawadi and Gaur, 1988; Khurana and Sharma, 2000).

A pot experiment was carried out on yield components of wheat (*Triticum aestivum*) to study the effects of single and combined inoculation of nitrogen fixing bacteria (*Rhizobium leguminosarum*) with PSB (*Pseudomonas* sp. strain 54RB). The shoot weight increased with *Rhizobium* inoculation along with fertilizer that was similar to pots with PSB fertilizer as well as combined inoculation of PSB and *Rhizobium* with fertilizer. Combined inoculation with fertilizer yielded maximum root weight (Afzal and Bano, 2008).

In soybean, pot experiments were conducted to evaluate the effects of *Rhizobium legiminosarum* strain alone and in combination with PSB (*Bacillus and Pseudomonas*). The plants grown with combination showed better pod filling and increased root and shoot weight suggesting a promising way for enhancing the growth of legume crops (Fatima *et al*., 2006). In another study, the combined effect of *Bradyrhizobium japonicum* and a PSB (*Pseudomonas sp.*) enhanced the number of nodules, dry weight of nodules, yield components, soil nutrient availability and uptake of the soybean crop (Tran *et al*., 2006).

Improved colonization, growth promotion and increase in phosphorous concentration were observed in lettuce and maize, on inoculation with two strains of *Rhizobium leguminosarum* and phosphate solubilizing bacteria, *Pseudomonas sp.* (Chabot *et al*., 1993). The single and combined inoculation of *Rhizobium* and PSB with fertilizer significantly increased the root and shoot weight, plant height, spike length, grain yield, seed P content, leaf protein and leaf sugar content of the wheat (*Triticum aestivum)* in a P-deficient natural non-sterilized sandy loam soil which was 30-40% better than application of P fertilizer alone for improving the grain yield in wheat crop. It was recommended that the phosphate solubilizing and the N_2-fixing bacterial strains had great potential in being formulated and used as biofertilizers (Cakmakc *et al*., 2007).

The effect of *Rhizoboum* and PSB (*Pseudomonas aeruginosa and Bacillus subtilis*) showed synergistic effect on symbiotic parameters and grain yield of Mungbean (Siddiqui *et al*., 2001). These PSB (*Pseudomonas aeruginosa and Bacillus subtilis)* bacterial strains improved the competitive ability and symbiotic effectiveness in Lentil (*Lens culinaris*) also under field

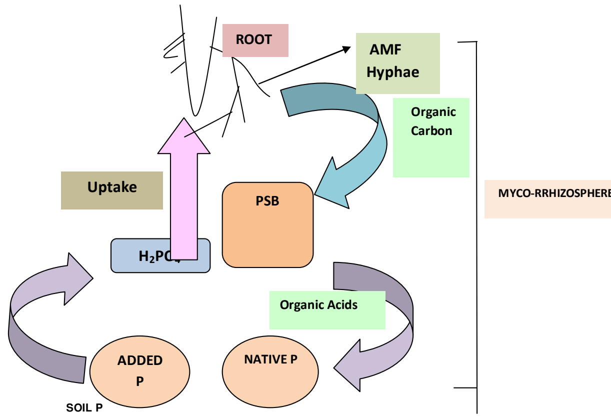

Figure 4. Solubilization of Phosphates in the Mycorrhizosphere and the mycorrhizal Phosphorous uptake

conditions on inoculation with *Rhizobium* (Kumar and Chandra, 2008). These observations indicated positive influence of combined application of *Rhizobium* and PSB in improving the growth and productivity of crop plants.

❖ PSB and Non-Symbiotic bacteria

There were several studies which demonstrated the beneficial effects of combined inoculation of PSB and non symbiotic bacteria *Azotobacter* on yield and nitrogen (N) and Phosphorous (P) accumulation of different crops (Monib *et al.,* 1984). For example, *Pseudomonas striata* and *Bacillus Polymyxa* with strains of *Azospirillum brasilense* resulted in significant improvement of grain and dry matter yields and N and P uptake, as compared to separate inoculations with each strain (Barea *et al.,* 1975). The combined inoculation of PSB and non-symbiotic bacteria like *Azospirillum, Azotobacter, Bacillucs* and *Acetobacter* has been explored and found to be beneficial for the crops. In *Cicer areitinum, Phaseolus mungo, Vigna* species and *Zea mays*, an increase in seed germination on inoculation with *Azospirillum* and *Pseudomonas* sp. was observed (Saikia and Bezbaruah, 1995).

Azotobacter, in combination with PSB had been shown to increase the yield of wheat upto 30% (Gholami et al., 2009; Kloepper et al., 1992) over control. Maximum nitrogenase activity was exhibited by *Azospirillum* isolates from sugarcane among *Bacillus*, *E.coli* and *Pseudomonas* species (Gangwar and Kaur, 2009). A PSB *Agrobacterium radiobacter* on combination with *Azospirillum lipoferum* produced improved grain yield of barley as compared to single inoculations in pot and field experiments (Belimov *et al.,* 1995).

An increase in growth and yield of pearl millet 'blackgrain' on inoculation with mixed inoculants of *Azospirillum lipoferum* and *Bacillus megaterium,* under pot culture conditions was observed which resulted in enhanced germination, seedling vigor, plant height, increased the nodulation and nitrogen fixation, seed weight and yield in blackgram (Poongguzhali *et al.,* 2005).

It was observed experimentally that there were number of metabolites which were released by *Bacillus* strains, that strongly affect the environment by increasing the nutrient availability of the plants (Barriuso and Solano, 2008). *Bacillus* sp. in combination with PSB was found to increase the growth, yield and nutrition of raspberry plant under organic growing conditions (Orhan *et al*., 2006). *Bacillus megaterium* and PSB were very consistent in improving the different root parameters like rooting performance, length, dry matter content of root in mint plants (Kaymak *et al.,* 2008).

❖ PSB and Vesicular-Arbuscular Fungi (VAM)

Vesicular arbuscular mycorrhiza (VAM) is mutualistic symbiont that is ubiquitous in roots of vascular plants in nature (Bajwa et al., 1999). There are various benefits of VAM in combination with phosphate solubilizing bacteria to plants since they impart nutrient absorption, stimulation of growth regulatory substances, osmotic adjustments under drought stress, enhancement of nitrogen fixation by symbiotic bacteria, increase resistance to soil pathogens and tolerance to environmental stresses (Bethlenfalvay and Linderman, 1992).

Symbiotic interaction was observed between PSB and VAM fungi, including the majority of those where plant growth promoting rhizobacteria and nitrogen fixing bacteria were involved (Meyer and Linderman, 1986b; von Alten *et al.,* 1993; Secillia and Bagyaraj, 1987; Bir'o *et al.,* 2000). These interactions were found to occur in the zone of soil surrounding the roots and fungal hyphae, which was commonly reffered to as 'mycorrizosphere' (Rambelli, 1973). Uptake of phosphorous from the soil is mediated by mycorrhizal fungi in addition to plant roots (Figure 4.). VAM in combination with PSB can improve the uptake of phosphorous and increase the crop production (Young *et al*., 1990). There are various soil fungi including VAM and bacteria which are helpful in solubilisation of inorganic phosphorous (Singal *et al.,* 1994). Some bacteria formed the synergetic interactions with VAM (Frey-Klett, 1997).

It was experimentally demonstrated that bridge was formed by VAM between roots and surrounding soils that increased the uptake of nutrients from the soil (Jeffries, 1994). PSB in combination with VAM exhibited a high efficiency to improve the plant growth and nutrition of alfalfa crop (Piccini and Azcon, 1987). Combined inoculation with *Bacillus circulans* and *Cladosporium herbarum* and VAM fungus resulted in the improved wheat (*Triticum aestivum*) crop yields in nutrient deficient soils (Singh and Kapoor, 1999). Effect of PSB (*Pseudomonas striata*) and VAM was studied on growth, yield and uptake by wheat and chickpea in field conditions. Single inoculation i.e. phosphorous alone, had no significant influence on yield of the crops, but the combined inoculation had shown the significant influence on growth, yield and uptake by wheat and chickpea (Mukherjee and Rai, 2000). VAM fungi (*Glomus fasciculatum*) and PSB (*Bacillus megaterium* var. phosphaticum) had shown the improved nodulation, nutrient uptake and phosphorous balance, mineral uptake, seed yield and available phosphorous in soil by Soybean (*Glycine max*) plant under field conditions (Dadhich *et al.,* 2006).

Arbuscular Mycorrizal fungi (AMF) constitute an integral part of terrestrial ecosystem and are ubiquitous in nature. They live in symbiotic relationship with roots of systems of over 80% of all terrestrial plant species, including many agronomically important species (Harrier and Waston, 2004). The beneficial traits of the PSB and fungi are studied separately. Synergistic effects of bacteria and AMF with respect to their combined beneficial impacts on plants have been observed (Artursson *et al*., 2006). AMF also have the ability to influence plant growth, water and nutrient content (Barea *et al*., 2002; Giovannetti *et al.,* 2006). They have the high affinity phosphate mechanism which enhances phosphorous nutrition in plants. It is observed in several studies that AMF have the ability to scavenge the available phosphorous by their hyphae having the large surface area on which extraradical hyphae act as a bridge between soil and the roots of the plants (Bianciotto and Bonfante, 2002).

AMF together with some specific bacteria i.e. PSB were known to create a more synergism, which was helpful in improved plant growth, including nutrient uptake in the plants (Barea, 1997; 2000), inhibition of pathogens and increased root branching (Budi *et al.,* 1999; Gamalero *et al.,* 2004). AM fungi were known to enhance plant uptake of mineral nutrients and this improved development led to disease escape and tolerance against soil borne pathogens (Bodker *et al.,* 1998; Dehne, 1982).

An experiment was carried out to evaluate the effects of nitrogen fixing bacteria (*Bradyrhizobium sp.*), PSB (*Bacillus subtilis*), phosphate solubilizing fungus (*Aspergillus awamori*) and AM fungus (*Glomus fasciculatum*) on the growth, chlorophyll content, seed yield and nodulation, N and P uptake of greengram plants which were grown in P deficient soils. The growth was promoted leading to improved yield of the greengram (Zaidi and Khan, 2006).

In another experiment, the interactive effects of PSB (*Bacillus polymyxa*), nitrogen fixing bacteria (*Azospirillum brasilense*) and arbuscular mycorrhizal fungus (*Glomus aggregatum*) were studied on palmasora (*Cymbopogon martini*), an aromatic grass, in a low phosphate alkaline soil amended with tri-calcium phosphate (TCP). These all microbes contributed as 'mycorrhiza helper'' and enhanced the root colonization. There was increase in growth and nutrient uptake of the plants by the combined inoculation of *Glomus aggretatum* and *Bacillus polymyxa*. The higher productivity of palmarosa plant was observed on combined inoculation of microbes (Ratti, 2001).

An experiment was carried on neem seedlings (*Azadirachta indica*) where the seedlings were inoculated with AM (*Glomus intraradices and Glomus geosporum*) and PSB (*Azospirillum brasilense*). The combined inoculation of microbes stimulated the growth (Muthukumar *et al*., 2001).

PSB as Biocontrol Agents

PSB and plant growth promoting rhizobacteria play an important role as biocontrol agents. They are indigenous to soil and plant rhizosphere. They suppress the activity of the wide range of the bacterial, fungal and nematode diseases. Use of phosphate solubilizing bacteria as environment friendly biofertilizer helps to reduce the much expensive phosphatic fertilizers (Park *et al*., 2010). In soil microenvironment, microbes play an important role as biocontrol agents. PGPR are of great importance in agriculture for the biocontrol of plants pathogens and biofertilization (Siddiqui, 2006).

Pseudomonas sp. is considered as the group of bacteria having the potential to act as the biocontrol agents (Kremer and Kennedy, 1996). In agricultural soils, they are ubiquitous. They secrete the various metabolites such as siderophores, gluconic acid and lytic enzymes which act as the biocontrol agents (Whipps, 2001). *Pseudomonas sp.* exhibit some of the traits that make them well suited to act as growth promoting and biocontrol agents (Weller, 1988) such as follows:.

- they grow rapidly
- they rapidly utilize seed and root exudates
- they colonize and multiply in rhizosohere
- they produce bioactive metabolites such as antibiotics, siderophores, volatiles
- adapted to environmental stresses

Bacillus subtilis is also used as a powerful biocontrol agent. It has the ability to produce endospores and produce biologically active compounds (Nagoraska *et al.*, 2007). *Bacillus megaterium* has ability to solubilize phosphorous and produce indole acetic acid (IAA), siderophores, and antifungal metabolites and reduces the disease intensity (Chakraborty *et al.,* 2006). PSB are used in integrated pest management. Application of PGPR is a possible way in agriculture for biocontrol of plant pathogens and biofertilization (Siddiqui, 2006).

CONCLUSIONS

P is an essential macronutrient, required for plant growth and is present in the soil in lesser amounts. P present in the soil is precipitated as orthophosphates and adsorbed mainly by Fe or Al oxides to become bioavailable by bacteria through their organic acid production and acid phosphatase secretion. This process is known as the mineral phosphate solubilisation (MPS). Soil has the high buffering capacity, due to which there is the reduction in the effectiveness of PSB in releasing P. The enhanced microbial activity through P solubilizing inoculants may contribute considerably in plant P uptake. PSBs mainly *Bacillus, Pseudomonas and Enterobacter* are very effective for increasing plant available P in soil as well as the growth and yield of various crop plants. Therefore, the use of the PSBs through bio-fertilization has enormous potential for making use of ever increasing fixed P in the soil and natural reserves of phosphate rocks. There is a need to explore PSB with greater efficiency and synergy with other microbes interacting with plants. More research is needed to explore the impact of PSB in affecting the various physiological, biochemical and molecular events governing the stimulation of growth by these microbes in the plants.

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