

Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production

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ABSTRACT

Plants acquire phosphorus from soil solution as phosphate anion. It is the least mobile element in plant and soil contrary to other macronutrients. It precipitates in soil as orthophosphate or is adsorbed by Fe and Al oxides through legend exchange. Phosphorus solubilizing bacteria play role in phosphorus nutrition by enhancing its availability to plants through release from inorganic and organic soil P pools by solubilization and mineralization. Principal mechanism in soil for mineral phosphate solubilization is lowering of soil pH by microbial production of organic acids and mineralization of organic P by acid phosphatases. Use of phosphorus solubilizing bacteria as inoculants increases P uptake. These bacteria also increase prospects of using phosphatic rocks in crop production. Greater efficiency of P solubilizing bacteria has been shown through co-inoculation with other beneficial bacteria and mycorrhiza. This article incorporates the recent developments on microbial P solubilization into classical knowledge on the subject.

Key words: Soil phosphorus, Solubilization, Mineralization, Organic acids, Soil pH, *Bacillus*, *Pseudomonas*.

INTRODUCTION

Phosphorus (P) is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa *et al.* 2002). Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition. Although microbial inoculants are in use for improving soil fertility during the last century, however, a meager work has been reported on P solubilization compared to nitrogen fixation. Soil P dynamics is characterized by physicochemical (sorption-desorption) and biological (immobilization-mineralization) processes. Large amount of P applied as fertilizer enters in to the immobile pools through precipitation reaction with highly reactive Al³⁺ and Fe³⁺ in acidic, and Ca²⁺ in calcareous or normal soils (Gyaneshwar *et al.*, 2002; Hao *et al.*, 2002). Efficiency of P fertilizer throughout the world is around 10 - 25 % (Isherword, 1998), and concentration of bioavailable P in soil is very low reaching the level of 1.0 mg kg⁻¹ soil (Goldstein, 1994). Soil microorganisms play a key role in soil P dynamics and subsequent availability of phosphate to plants (Richardson, 2001).

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 i.e. PO₄³⁻ directly, releasing P into solution (He *et al.*, 2002). Phosphate solubilizing bacteria (PSB) are being used as biofertilizer since 1950s (Kudashev, 1956; Krasilinikov, 1957). Release of P by PSB from insoluble and fixed / adsorbed forms is an import aspect regarding P availability in soils. There are strong evidences that soil bacteria are capable of transforming soil P to the forms available to plant. Microbial biomass assimilates soluble P, and prevents it from adsorption or fixation (Khan and Joergesen, 2009). Microbial community influences soil fertility through soil processes viz. decomposition, mineralization, and storage / release of nutrients. Microorganisms enhance the P availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphates (Chen *et al.*, 2006; Kang *et al.*, 2002; Pradhan and Sukla, 2005). These bacteria in the presence of labile carbon serve as a sink for P by rapidly immobilizing it even in low P soils (Bünemann *et al.*, 2004). Subsequently, PSB become a source of P to plants upon its

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release from their cells. The PSB and plant growth promoting rhizobacteria (PGPR) together could reduce P fertilizer application by 50 % without any significant reduction of crop yield (Jilani *et al.*, 2007; Yazdani *et al.*, 2009). It infers that PSB inoculants / biofertilizers hold great prospects for sustaining crop production with optimized P fertilization.

Phosphorus Solubilizing Microorganisms

Evidence of naturally occurring rhizospheric phosphorus solubilizing microorganism (PSM) dates back to 1903 (Khan *et al.*, 2007). Bacteria are more effective in phosphorus solubilization than fungi (Alam *et al.*, 2002). Among the whole microbial population in soil, PSB constitute 1 to 50 %, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5 % in P solubilization potential (Chen *et al.*, 2006). Number of PSB among total PSM in north Iranian soil was around 88 % (Fallah, 2006). Microorganisms involved in phosphorus acquisition include mycorrhizal fungi and PSMs (Fankem *et al.*, 2006). Among the soil bacterial communities, ectorrhizospheric strains from *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia have been described as effective phosphate solubilizers (Iguar *et al.*, 2001). Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi are the most powerful P solubilizers (Whitelaw, 2000). *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, and *Enterobacter* could be referred as the most important strains (Subbarao, 1988; Kucey *et al.*, 1989). A nematofungus *Arthrobotrys oligospora* also has the ability to solubilize the phosphate rocks (Duponnois *et al.*, 2006).

Occurrence of Phosphate Solubilizing Bacteria

High proportion of PSM is concentrated in the rhizosphere, and they are metabolically more active than from other sources (Vazquez *et al.*, 2000). Usually, one gram of fertile soil contains 10^1 to 10^{10} bacteria, and their live weight may exceed 2,000 kg ha⁻¹. Soil bacteria are in cocci (sphere, 0.5 µm), bacilli (rod, 0.5–0.3 µm) or spiral (1-100 µm) shapes. Bacilli are common in soil, whereas spirilli are very rare in natural environments (Baudoin *et al.* 2002). The PSB are ubiquitous with variation in forms and population in different soils. Population of PSB depends on different soil properties (physical and chemical properties, organic matter, and P content) and cultural activities (Kim *et al.*, 1998). Larger populations of PSB are found in agricultural and rangeland soils (Yahya and Azawi, 1998). In north of Iran, the PSB count ranged from 0 to 107 cells g⁻¹ soil, with 3.98 % population of PSB among total bacteria (Fallah, 2006).

Mechanisms of Phosphorus Solubilization

Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively (Hilda and Fraga, 2000; Khiari and Parent, 2005). Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe *et al.*, 1998). Phosphate solubilization takes place through various microbial processes / mechanisms including organic acid production and proton extrusion (Surange, 1995; Dutton and Evans, 1996; Nahas, 1996). General sketch of P solubilization in soil is shown in Figure 1. A wide range of microbial P solubilization mechanisms exist in nature, and much of the global cycling of insoluble organic and inorganic soil phosphates is attributed to bacteria and fungi (Banik and Dey, 1982). Phosphorus solubilization is carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms (Whitelaw, 2000). Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils (Kpombekou and Tabatabai,

1994; Stevenson, 2005). The PSB dissolve the soil P through production of low molecular weight organic acids mainly gluconic and keto gluconic acids (Goldstein, 1995; Deubel *et al.*, 2000), in addition to lowering the pH of rhizosphere. The pH of rhizosphere is lowered through biotical production of proton / bicarbonate release (anion / cation balance) and gaseous (O_2/CO_2) exchanges. Phosphorus solubilization ability of PSB has direct correlation with pH of the medium.

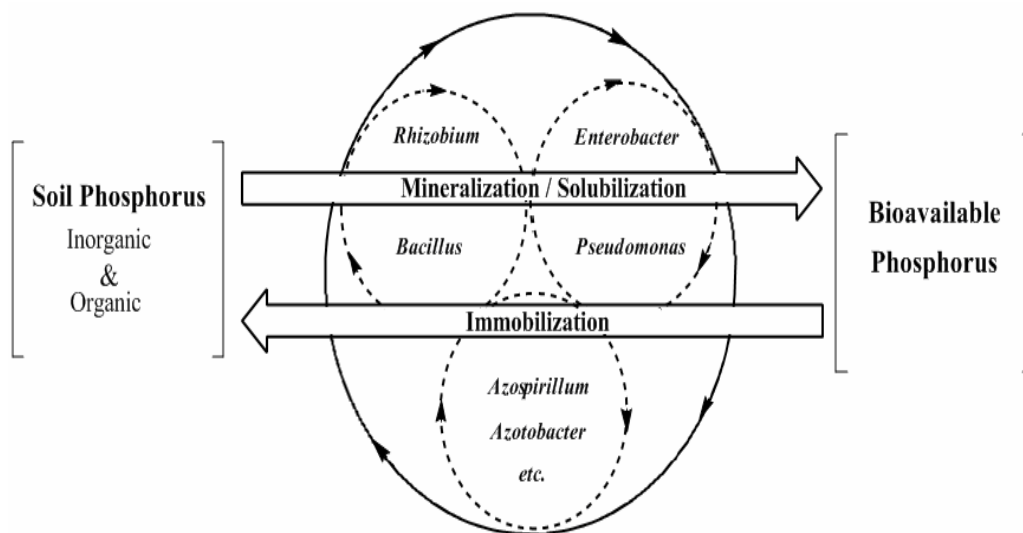


Figure 1. Schematic diagram of soil phosphorus mobilization and immobilization by bacteria

Release of root exudates such as organic ligands can also alter the concentration of P in the soil solution (Hinsinger, 2001). Organic acids produced by PSB solubilize insoluble phosphates by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil (Nahas, 1996). Inorganic acids e.g. hydrochloric acid can also solubilize phosphate but they are less effective compared to organic acids at the same pH (Kim *et al.*, 1997). In certain cases phosphate solubilization is induced by phosphate starvation (Gyaneshwar *et al.*, 1999).

Solubilization of Ca-bound P

Soil phosphates mainly the apatites and metabolites of phosphatic fertilizers are fixed in the form of calcium phosphates under alkaline conditions. Many of the calcium phosphates, including rock phosphate ores (fluoroapatite, francolite), are insoluble in soil with respect to the release of inorganic P (P_i) at rates necessary to support agronomic levels of plant growth (Goldstein, 2000). Gerretsen (1948) first showed that pure cultures of soil bacteria could increase the P nutrition of plants through increased solubility of Ca-phosphates. 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). Microorganisms through secretion of different types of organic acids e.g. carboxylic acid (Deubel and Merbach, 2005) and rhizospheric pH lowering mechanisms (He and Zhu, 1988) dissociate the bound forms of phosphate like $Ca_3(PO_4)_2$. Nevertheless, buffering capacity of the medium reduce the effectiveness of PSB in releasing P from tricalcium phosphates (Stephen and Jisha, 2009).

Acidification of the microbial cell surroundings releases P from apatite by proton substitution / excretion of H^+ (accompanying greater absorption of cations than anions) or release of Ca^{2+} (Goldstein, 1994; Illmer and Schinner 1995; Villegas and Fortin 2002). While, the reverse

occurs when uptake of anions exceeds that of cations, with excretion of $\text{OH}^- / \text{HCO}_3^-$ exceeding that of H^+ (Tang and Rengel, 2003). Carboxylic anions produced by PSB, have high affinity to calcium, solubilize more phosphorus than acidification alone (Staunton and Leprince 1996). Complexing of cations is an important mechanism in P solubilization if the organic acid structure favors complexation (Fox *et al.*, 1990). It is controlled by nutritional, physiological and growth conditions of the microbial culture (Reyes *et al.*, 2007), but it is mostly due to the lowering of pH alone by organic acids (Moghimi and Tate, 1978) or production of microbial metabolites (Abd-Alla, 1994). Organic anions and associated protons are effective in solubilizing precipitated forms of soil P (e.g. Fe - and Al - P in acid soils, Ca - P in alkaline soils), chelating metal ions that may be associated with complexed forms of P or may facilitate the release of adsorbed P through ligand exchange reactions (Jones, 1998). Calcium phosphate (Ca-P) release results from the combined effects of pH decrease and carboxylic acids synthesis, but proton release cannot be the single mechanism (Deubel *et al.*, 2000).

Table 1. Phosphorus solubilization potential of various bacterial strains at different pH values of the medium

PSB strains	Estimated quantity in a standing liquid culture		
	P ($\mu\text{g mL}^{-1}$)	pH	References
D 5/23 <i>Pantoea agglomerans</i>	62.76	5.93	Amellal <i>et al.</i> (1999)
CC 322 <i>Azospirillum</i> sp. Mac 27	83.39	6.19	Ratti <i>et al.</i> (2001)
<i>Azotobacter chroococcum</i> Ala 27	98.11	4.84	Narula <i>et al.</i> (2000)
<i>Azotobacter chroococcum</i> Msx 9	1.10	7.50	Narula <i>et al.</i> (2000)
<i>Azotobacter chroococcum</i> ER 3	65.90	5.82	Narula <i>et al.</i> (2000)

Solubilization of Al- / Fe-bound P

Solubilization of Fe and Al occurs via proton release by PSB by decreasing the negative charge of adsorbing surfaces to facilitate the sorption of negatively charged P ions. Proton release can also decrease P sorption upon acidification which increases H_2PO_4^- in relation to HPO_4^{2-} having higher affinity to reactive soil surfaces (Whitelaw, 2000). Carboxylic acids mainly solubilize Al-P and Fe-P (Henri *et al.*, 2008; Khan *et al.*, 2007) through direct dissolution of mineral phosphate as a result of anion exchange of PO_4^{3-} by acid anion, or by chelation of both Fe and Al ions associated with phosphate (Omar, 1998). It is through root colonizing pseudomonads with high-affinity iron uptake system based on the release of Fe^{3+} - chelating molecules i.e. siderophores (Altomare, 1999). Moreover, carboxylic anions replace phosphate from sorption complexes by ligand exchange (Otani *et al.*, 1996; Whitelaw, 2000) and chelate both Fe and Al ions associated with phosphate, releasing phosphate available for plant uptake after transformation. Ability of organic acids to chelate metal cations is greatly influenced by its molecular structure, particularly by the number of carboxyl and hydroxyl groups. Type and position of the ligand in addition to acid strength determine its effectiveness in the solubilization process (Kpombekou and Tabatabai, 1994). Phosphorus desorption potential of different carboxylic anions lowers with decrease in stability constants of Fe - or Al - organic acid complexes ($\log K_{\text{Al}}$ or $\log K_{\text{Fe}}$) in the order: citrate > oxalate > malonate / malate > tartrate > lactate > gluconate > acetate > formiate (Ryan *et al.* 2001).

Mineralization of organic P

Mineralization of soil organic P (Po) plays an imperative role in phosphorus cycling of a farming system. Organic P may constitute 4-90 % of the total soil P. Almost half of the microorganisms in soil and plant roots possess P mineralization potential under the action of phosphatases (Cosgrove, 1967; Tarafdar *et al.*, 1988). Alkaline and acid phosphatases use organic phosphate as a substrate to convert it into inorganic form (Beech *et al.*, 2001). Principal mechanism for mineralization of soil organic P is the production of acid phosphatases (Hilda and

Fraga, 2000). Release of organic anions, and production of siderophores and acid phosphatase by plant roots / microbes (Yadaf and Tarafdar, 2001) or alkaline phosphatase (Tarafdar and Claasen, 1988) enzymes hydrolyze the soil organic P or split P from organic residues. The largest portion of extracellular soil phosphatases is derived from the microbial population (Dodor and Tabatabai, 2003). *Enterobacter agglomerans* solubilizes hydroxyapatite and hydrolyze the organic P (Kim *et al.*, 1998). Mixed cultures of PSMs (*Bacillus*, *Streptomyces*, *Pseudomonas* etc.) are most effective in mineralizing organic phosphate (Molla *et al.*, 1984).

Interaction of PSB with other Microorganism

Symbiotic relationship between PSB and plants is synergistic in nature as bacteria provide soluble phosphate and plants supply root borne carbon compounds (mainly sugars), that can be metabolized for bacterial growth (Pérez *et al.*, 2007). The PSM along with other beneficial rhizospheric microflora enhance crop production. Simultaneous application of *Rhizobium* with PSM (Perveen *et al.*, 2002) or arbuscular mycorrhizae (AM) fungi (Zaidi *et al.*, 2003) has been shown to stimulate plant growth more than with their sole inoculation in certain situations when the soil is P deficient. Synergistic interactions on plant growth have been observed by co-inoculation of PSB with N₂ fixers such as *Azospirillum* (Belimov *et al.*, 1995) and *Azotobacter* (Kundu and Gaur, 1984), or with vesicular arbuscular mycorrhizae (Kim *et al.*, 1998).

Amount of Solubilized P

The PSB solubilize the fixed soil P and applied phosphates resulting in higher crop yields (Gull *et al.*, 2004). Direct application of phosphate rock is often ineffective in the short time period of most annual crops (Goenadi *et al.*, 2000). Acid producing microorganisms are able to enhance the solubilization of phosphatic rock (Gyaneshwar *et al.*, 2002). The PSB strains exhibit inorganic P-solubilizing abilities ranging between 25–42 $\mu\text{g P mL}^{-1}$ and organic P mineralizing abilities between 8–18 $\mu\text{g P mL}^{-1}$ (Tao *et al.*, 2008). The PSB in conjunction with single super phosphate and rock phosphate reduce the P dose by 25 and 50 %, respectively (Sundara *et al.*, 2002). *Pseudomonas putida*, *P. fluorescens* Chao and *P. fluorescens* Tabriz released 51, 29 and 62 % P, respectively; with highest value of 0.74 mg P / 50 mL from Fe₂O₃ (Ghaderi *et al.*, 2008). *Pseudomonas striata* and *Bacillus polymyxa* solubilized 156 and 116 mg P L⁻¹, respectively (Rodríguez and Fraga, 1999). *Pseudomonas fluorescens* solubilized 100 mg P L⁻¹ containing Ca₃(PO₄)₂ or 92 and 51 mg P L⁻¹ containing AlPO₄ and FePO₄, respectively (Henri *et al.*, 2008).

Effect of PSB on Crop Production

Phosphate rock minerals are often too insoluble to provide sufficient P for crop uptake. Use of PSMs can increase crop yields up to 70 percent (Verma, 1993). Combined inoculation of arbuscular mycorrhiza and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock (Goenadi *et al.*, 2000; Cabello *et al.*, 2005). Higher crop yields result from solubilization of fixed soil P and applied phosphates by PSB (Zaidi (1999). Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixation (Kucey *et al.*, 1989; Ponmurugan and Gopi, 2006). *Pseudomonas* spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (Son *et al.*, 2006). Phosphate solubilizing bacteria enhanced the seedling length of *Cicer arietinum* (Sharma *et al.*, 2007), while co-inoculation of PSM and PGPR reduced P application by 50 % without affecting corn yield (Yazdani *et al.*, 2009). Inoculation with PSB increased sugarcane yield by 12.6 percent (Sundara *et al.*, 2002). Sole application of bacteria increased the biological yield, while the application of the same bacteria along with mycorrhizae achieved the maximum grain weight

(Mehrvarz *et al.*, 2008). Single and dual inoculation along with P fertilizer was 30-40 % better than P fertilizer alone for improving grain yield of wheat, and dual inoculation without P fertilizer improved grain yield up to 20 % against sole P fertilization (Afzal and Bano, 2008). Mycorrhiza along with *Pseudomonas putida* increased leaf chlorophyll content in barley (Mehrvarz *et al.*, 2008). Rhizospheric microorganisms can interact positively in promoting plant growth, as well as N and P uptake. Seed yield of green gram was enhanced by 24 % following triple inoculation of *Bradyrhizobium* + *Glomus fasciculatum* + *Bacillus subtilis* (Zaidi and Khan, 2006). Growth and phosphorus content in two alpine *Carex* species increased by inoculation with *Pseudomonas fortinii* (Bartholdy *et al.*, 2001). Integration of half dose of NP fertilizer with biofertilizer gives crop yield as with full rate of fertilizer; and through reduced use of fertilizers the production cost is minimized and the net return maximized (Jilani *et al.*, 2007).

CONCLUSION

Soil P precipitated as orthophosphate and adsorbed by Fe and Al oxides is likely to become bio-available by bacteria through their organic acid production and acid phosphatase secretion. Although, high buffering capacity of soil reduces the effectiveness of PSB in releasing P from bound phosphates; however, enhancing microbial activity through P solubilizing inoculants may contribute considerably in plant P uptake. Phosphorus solubilizing bacteria mainly *Bacillus*, *Pseudomonas* and *Enterobacter* are very effective for increasing the plant available P in soil as well as the growth and yield of crops. So, exploitation of phosphate solubilizing bacteria through biofertilization has enormous potential for making use of ever increasing fixed P in the soil, and natural reserves of phosphate rocks.

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