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# Phosphate Solubilizing Bacteria for a Sustainable Agriculture – A Review

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## Abstract

Phosphorus is a major, essential macronutrient required for plant growth and development. Most soils contain insoluble inorganic phosphates, but they are of no use to plants unless they are solubilized. Therefore, in order to maintain the amount of phosphorus available in soil for plant use, a large amount of phosphorus-based fertilizer is often added to soil, the bulk of which could also be converted to insoluble form. This makes continuous application necessary, which in turn pollutes the soil. To overcome this problem, bacterial inoculants are an important approach that increases plant production for sustainable development. Microorganisms like phosphate-solubilizing bacteria isolated from different plants are found to solubilize the insoluble phosphates. There are different mechanisms involved to solubilize the insoluble phosphate into the soluble form and make it available to the plants, either by lowering the pH, organic acid production, the involvement of enzymes, etc. Application of phosphate-solubilizing bacteria not only solubilizes the insoluble phosphates, but they are also found to increase plant growth. In this review, we have focused on the importance of phosphorus, types of PSB, mechanisms to solubilize insoluble phosphates, and the effect of PSB on plant growth.

***Key words*: Phosphorus, PSB, pH, Organic acid, Enzymes, Plant growth**

Phosphorus is one of the major plant nutrients, second only to nitrogen in requirement and makes up about 0.2% of plant dry weight [1]. It plays an important role in all major metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration [2] and nitrogen fixation in legumes [3]. A greater part of soil phosphorus, approximately 95-99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants [4]. Up to 75% of the soluble phosphate fertilizers added to crops may be converted to sparingly soluble forms by reacting with the free $Ca^{2+}$ ions in high pH soils or with $Fe^{3+}$ and $Al^{3+}$ in low pH soils [5-6]. To increase the availability of phosphorus for plants, large amounts of fertilizer are used on a regular basis. But after application, a large proportion of fertilizer phosphorus is quickly transferred to the insoluble form [7]. Therefore, very little percentage of the applied phosphorus is used, making continuous application necessary [8]. Plants can absorb phosphate in two soluble forms, the monobasic ($H_2PO_4^-$) and the dibasic ($HPO_4^{2-}$) ions [9]. Interest has been focused on the inoculation of phosphate-solubilizing micro-organisms into the soil so as to increase the availability of native fixed phosphate and to reduce the use of fertilizers [10].

Phosphate solubilising microorganisms are capable of solubilizing tricalcium, aluminium and iron phosphates, as well as rock phosphate making the phosphorus present in the soil available to the plants [11-12]. Soils also contain organic phosphorus, which can be used by crops only if it is mineralized. Organisms that cause increases in plant available phosphate in the soil system belong to a diversified group including bacteria, actinomycetes and several groups of fungi. The composition and dynamics of this functional group was influenced greatly by vegetation type, soil texture, soil chemical elements, and pH in soil solution [13-15]. It was reported that about 20% of microorganisms in soil can solubilize insoluble inorganic phosphate and that phosphate solubilizing activity of PSM is related to the environmental conditions such as farming practices. Phosphate solubilizing bacteria are common in the rhizosphere and secretion of organic acids and phosphatases are common method of facilitating conversion of insoluble forms of phosphate to plant-available forms [16]. Phosphate solubilizing bacteria have been used to convert insoluble rock phosphate into soluble form and make it available for the plant growth [17-18]. This conversion is through acidification [19-20], chelation and exchange reactions [21-23] and produces, in the periplasm, strong organic acids [24], which have become indicators for routine isolation and selection procedures of phosphate solubilizing bacteria [25].

PSB also produce amino acids, vitamins and growth promoting substances [25-26], which promote plants growth. It was reported that IAA produced by bacteria improves plant growth by increasing the number of root hairs and lateral roots [27]. The production of Indole Acetic Acid (IAA), gibberellins and cytokinins by PSB has been reported earlier by several workers [28]. Many PSB are proved to be effective biofertilizers or bio-controlling agents and can be regarded as broad spectrum biofertilizers [29]. These findings have been further supported by many researchers. Likewise, Chakraborty *et al*. [30] reported that *Bacillus megaterium* promoted the

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growth of tea seedlings and two-year old plants significantly, as evidenced by increased plant height, number of branches and leaves.

*Phosphate solubilizing bacteria*

The immediate vicinity of root surface which constitute the rhizosphere is an extremely important habitat for microbes. Roots secrete a number of compounds into the soil which may either enhance or inhibit the growth of microorganisms [31]. Kobus [32] reported that the numbers of PS bacteria in a soil were influenced more by soil type and the manner of its cultivation than by the physical composition or content of humus, N or P in the soil.

Bacteria are more effective in phosphorus solubilization than fungi [33]. 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 [34]. Among the soil bacterial communities, ectorhizospheric strains from *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia have been described as effective phosphate solubilizes [35]. Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* are the most powerful P solubilizers [36] (Whitelaw, 2000). *Bacillus megaterium*, *B. circulans*, *B.subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, and *Enterobacter* could be referred as the most important strains[37-38]. The most important P solubilizing bacterial genera are *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium and Erwinia* [39]. There are reports of other bacteria as P solubilizers such as *Azotobacter* [40], *Xanthomonas* [41], *Kurthia* [42] *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Phyllobacterium*, etc. [43], *Pantoea*, *Klebsiella* [44].

*Isolation of phosphate solubilizing bacteria*

Ten gram (10 g) of Rhizospheric soil sample was suspended in 90 ml of sterile distilled water and $10^{-1}$ dilution was obtained. Serial dilutions were prepared by mixing 1 ml of the suspension made into 9 ml sterile water blanks, until the 10-7 dilution was obtained. Pikovskaya's agar (10g Glucose, 5g tricalcium phosphate, 0.5g ammonium sulphate, 0.2g potassium sulphate, 0.1 g magnesium sulphate, 0.5 g yeast extract, trace amount of manganese sulphate and ferrous sulphate, 20 g agar, 1000 ml distilled water) medium was used for isolation and maintenance of PSB [45]. The serially diluted soil suspensions were spread-plated on Pikovskaya's agar plates and incubated at 37 °C for 7 days. Bacterial colonies causing clear zones by a turbid white background were considered as phosphate solubilizers. The diameter of PSB colony as well as halo zones were measured by using metric scale.

Fig 1 Bacterial colonies showing clear zones in Pikovskaya's Agar media [46]

*Mechanism of phosphate solubilization*

The main P solubilization mechanisms employed by soil microorganisms include: (1) release of complex or mineral dissolving compounds e.g., organic acid anions, siderophores, protons, hydroxyl ions, $CO_2$; (2) liberation of extracellular enzymes (biochemical P mineralization) and (3) the release of P during substrate degradation (biological P liberalization) [47]. Thus, microorganisms play an important role in the soil P cycle i.e., dissolution-precipitation, sorption-desorption and mineralization-immobilization.

Several theories exist explaining the mechanisms of microbial P solubilization: the sink theory [48], the organic acid theory [49], and the acidification by $H^+$ excretion theory [50].

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 [51-52]. The PSB dissolve the soil P through production of low molecular weight organic acids mainly gluconic and keto gluconic acids [53-54], in addition to lowering the pH of rhizosphere. Other organic acids that have been involved in the phosphate solubilization are primarily citric, lactic, gluconic, 2-ketogluconic, oxalic, glycolic, acetic, malic, fumaric, succinic, tartaric, malonic, glutaric, pro-pionic, butyric, glyoxylic, and adipic acid [55-60].

Among these organic acids, gluconic acid ranks as the most important in this process and has been studied widely [61-62].

A direct correlation between drops in pH and increase in available P of the culture media has been observed in certain cases [63-64]. In few others, the degree of solubilization was not always proportional to the decline in pH [65-66].

The workers who believe in organic acid theory hardly observed any correlation between the amount of P solubilized and organic acid concentration in the culture medium. Hence it is doubtful as to whether the organic acids are directly and exclusively involved in solubilization [67-68]. Solubilization of calcium phosphate has been reported to occur even in the absence of organic acid [10]. Banik and Dey [69] and Asea *et al.* [67] detected organic acids in culture solutions of PSM but did not show any correlation between the solubilization of P and amount of organic acids produced by PSM. However, an HPLC analysis of the culture solution of *Pseudomonas*, solubilized unavailable forms of P without any organic acid production [25]. In each of these cases, acidification of the medium resulted and it was postulated that $H^+$ excretion originating from $NH_4$ assimilation [68] and respiratory $H_2CO_3$ production [70] as an alternate mechanism of mineral phosphate solubilization. It was hypothesized that the gluconic acid produced lead to the release of protons that finally solubilize the insoluble phosphates [71]. In a study of *Pseudomonas fluorescens*, the form of C supply (e.g., glucose versus fructose) rather than N supply (e.g., $NH^{4+}$ versus $NO^{3-}$) had the greatest effect on proton release [72].

Rudolph [73], reported that MPS activity occurs as a consequence of microbial sulphur oxidation, nitrate production and $CO_2$ formation. These processes result in the formation of inorganic acids like sulphuric acid [22]. Further, Azam and Memon [74], supported their findings by saying that, bacteria like *Nitrosomonas* and *Thiobacillus* species can dissolve phosphate compounds by producing inorganic acids such as nitric and sulphuric acids. Inorganic acids e.g., hydrochloric acid can also solubilize phosphate but they are less effective compared to organic acids at the same pH [75]. However, the concept of involvement of inorganic acids in P solubilization are less effective than organic acids [75].

The major source of organic phosphorus in soil is the organic matter. Organic P may constitute 4-90% of the total soil P [76] and soil organic P is largely in the form of inositol phosphate (soil phytate). Other organic P compounds that have been reported are: phosphomonoesters, phosphodiesters, phospholipids, nucleic acids and phosphor-triesters [77]. According to the sink theory of Halvorson *et al*. [78]; P solubilizing organisms stimulate the indirect dissolution of Ca-P compounds by continuous removal of P.

Such P can be released from organic compounds in soil by three groups of enzymes: (1) Non-specific acid phosphatases (NSAPs): the most studied among these NSAPs enzymes released by PSM, are the phosphor-monoesterases also referred as phosphatases [79].

(2) Phytases: Another enzyme produced by PSM which is responsible for the release of P from phytate degradation. Phytate in its basic form is the primary source of inositol and are stored in the plant seeds and pollen [80],

(3) Phosphonatases and C-P lyases are able to release free P from recalcitrant organic P forms [81].

The overall results of the study indicate that acid production was not the only reason for phosphate solubilization. However, P-solubilization is a complex phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the culture [82] and in certain cases it is induced by phosphate starvation [83].

*Effects of PSB in plant growth*

Inappropriate application of mineral fertilizers in agriculture has resulted in pollution and salinization of agricultural lands and water resources. In particular, plant growth-promoting rhizobacteria (PGPR) have been reported to be key elements for plant establishment under nutrient-imbalance conditions. Their use in agriculture can favour a reduction in agro-chemical use and support ecofriendly crop production [84-86]. PGPR can help the improvement of plant growth, plant nutrition, root growth pattern, plant competitiveness, and responses to external stress factors. They can also inhibit soil borne plant pathogens by producing growth-promoting chemical substances and inducing plant resistance [87-89]. Different plant-growth promoting rhizosphere bacteria, including associative bacteria such as *Azospirillum*, *Bacillus*, *Pseudomonas*, *Enterobacter* group have been used for their beneficial effects on plant growth [90]. Several studies clearly showed the effect of plant growth-promoting bacteria on growth of different crops at different climates, soils and temperatures [91-92].

Phosphate solubilizing microorganisms have an important contribution to overall plant P nutrition and growth, and have increased yields of many crops [93].

Many researchers have reported an increase in P uptake and seed yields, due to PSB inoculation of wheat, barley, mungbean, chickpea and maize genotypes [94-95]. Increased in the plant P uptake and production by 34% in Maize by the application of plant growth-promoting bacteria (PGPB) such as *Azospirillum brasilense*, *Bacillus subtilis*, and *Pseudomonas fluorescens* have been reported by Pereira *et al*. [96]. Phosphate Solubilizing *Pseudomonas* and *Bacillus* species were inoculated into wheat resulting in improved phosphorus uptake and grain production [97]. Similarly, increased plant growth and phosphate uptake have been reported in many crop species as a result of PSB inoculants, e.g., *Pseudomonas* sp. in rice [98], *Pseudomonas* in soya bean [99] and *Pseudomonas* sp. in wheat [100]. *Rhizobium leguminosarum* is of particular interest because of its dual function: its ability to fix N and to solubilize P [101-103]. Inoculation with two strains of P solubilizing *R. leguminosarum* improved root colonization and growth in lettuce and maize. Additionally, rhizobia exhibited an ability to promote plant growth in non-legumes [104]. Increased in plant height, green fodder yield and grain yield of sorghum were reported by the application of PSB based biofertilizer [105].

Indirect growth promotion by PSM is achieved by reducing pathogen infection via the antibiotic or siderophores which are synthesized and supplied by the bacteria [106-107]. A rhizospheric bacterium *Pseudomonas fluorescens*, solubilizes P, and produces antibiotics such as pyoluteorin [108]. Hydrogen cyanide produced by *Pseudomonas* was used as a biological control of black root rot of tobacco [106].

Similarly, a number of *Pseudomonas* strains are found to be well adapted to higher altitude soils and have exhibited antifungal, phosphate solubilizing, and plant growth promoting properties [109]. These species have been reported as efficient degraders of organic matter [110-111].

## CONCLUSION

Out of nitrogen, phosphorus and potassium, phosphorus is the second essential macronutrient for plant growth and development. In spite of its presence in soil in large quantities, it is not easily available to the plants because of its fixation, which makes them insoluble. Due to the continuous application of chemical fertilizer to the soil, it has become necessary to find an alternative for the sustainable agriculture. Phosphate solubilizing bacteria present in the soil solubilize the insoluble phosphates and increase the plant yield. These properties of PSB are under consideration for use as a biofertilizer. However, further investigations are required to develop PSB as biofertilizers isolated from different crops, different geographical regions for better crop productivity and to reduce environmental pollution to promote sustainable agriculture.

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