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Review Article

Phosphate Solubilizing Microorganisms: Promising Approach as Biofertilizers

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Phosphorus (P) is a macronutrient required for the proper functioning of plants. Because P plays a vital role in every aspect of plant growth and development, deficiencies can reduce plant growth and development. Though soil possesses total P in the form of organic and inorganic compounds, most of them remain inactive and thus unavailable to plants. Since many farmers cannot afford to use P fertilizers to reduce P deficits, alternative techniques to provide P are needed. Phosphate solubilizing microbes (PSMs) are a group of beneficial microorganisms capable of hydrolyzing organic and inorganic insoluble phosphorus compounds to soluble P form that can easily be assimilated by plants. PSM provides an ecofriendly and economically sound approach to overcome the P scarcity and its subsequent uptake by plants. Though PSMs have been a subject of research for decades, manipulation of PSMs for making use of increasing fixed P in the soil and improving crop production at the field level has not yet been adequately commercialized. The purpose of this review is to widen the understanding of the role of PSMs in crop production as biofertilizers.

1. Introduction

Phosphorus (P) is one of the major growth-limiting macronutrients required for proper plant growth, particularly in tropical areas, due to its low availability in the soil [1]. It accounts for between 0.2 and 0.8% of the dry weight of plants [2], and it is contained within nucleic acids, enzymes, coenzymes, nucleotides, and phospholipids. P is essential in every aspect of plant growth and development, from the molecular level to many physiological and biochemical plant activities including photosynthesis [2], development of roots, strengthening the stalks and stems, formation of flowers and seeds, crop maturity and quality of crop, energy production, storage and transfer reactions, root growth, cell division and enlargement, N fixation in legumes, resistance to plant diseases [2–6], transformation of sugar to starch, and transporting of the genetic traits [5, 7]. Adequate P availability is also required for laying down the primordia of plant reproductive parts during the early phases of plant development [5].

Phosphorus is the second most important macronutrient required by the plants, next to nitrogen. Yet, the availability of soluble forms of P for plants in the soils is limited because of its fixation as insoluble phosphates of iron, aluminum, and calcium in the soil [2, 6–8]. Most soils possess considerable amounts of P, but a large proportion is bound to soil constituents. Soil with low total P can be supplemented with P fertilizer but are not able to hold the added P. About 75–90% of the added chemical P fertilizer is precipitated by metal-cation complexes and rapidly becomes fixed in soils and has long-term impacts on the environment in terms of eutrophication, soil fertility depletion, and carbon footprint [2].

Microorganisms are integral in the natural phosphorus cycle. The use of phosphate solubilizing microorganisms (PSMs) as biofertilizers for agriculture enhancement has been a subject of study for years. This review is intended to provide a brief on availability of soil P and diversity of PSM, mechanisms of P solubilization, how PSM induce plant growth, and their possible role as biofertilizer in crop production.

2. Availability of Phosphorus in the Soil

Phosphorus is a reactive element and does not exist as elemental form in the soil. Phosphorus in the soil solution exists as insoluble inorganic phosphorus and insoluble organic phosphorus [6]. Its cycle in the biosphere can be described as "sedimentary," because there is no interchange with the atmosphere, and unlike the case for nitrogen, no large atmospheric source can be made biologically available [6, 9]. Consequently, deficiency of phosphorus severely restricts the growth and yield of crops [6].

The phosphorus level in the soil is about 0.05% [2, 6]. Soil test values are generally much higher, but the greater part of it, about 95 to 99%, is present in the form of insoluble phosphates [10]. The concentration of soluble P in soil solution is usually very low, normally at levels varying from ppb in very poor soils to 1 mg/L in heavily fertilized soils [2, 4, 6, 9].

Plant cell might take up several P forms, but the greatest part is absorbed in the forms of phosphate anions mainly HPO_4^{2-} or $H_2PO_4^-$ depending upon soil pH [3, 5, 6, 9, 11].

The main input of inorganic P in agricultural soil is applying phosphorus fertilizers. Nearly, 70 to 90% of phosphorus fertilizers applied to soils is fixed by cations and converted inorganic P [6]. P gets immobilized by cations such as Ca^{2+} in calcareous or normal soils to form a complex calcium phosphate ($Ca_3(PO_4)_2$) and with Al^{3+} and Fe^{3+} in acidic soils to form aluminum phosphate (AlPO) and ferrous phosphate (FePO) [3, 5]. These are insoluble forms and consequently unavailable. These accumulated phosphates in agricultural soils are adequate to maintain maximum crop yields worldwide for about 100 years [6] if it could be mobilized, converted into soluble P forms using of PSM. A greater concern has, therefore, been made to get an alternative system yet low-priced technology that could supply adequate P to plants.

3. Diversity of Phosphate Solubilizing Microorganisms

Phosphate solubilizing microorganisms (PSMs) are group of beneficial microorganisms capable of hydrolyzing organic and inorganic phosphorus compounds from insoluble compounds. Among these PSMs, strains from bacterial genera (*Bacillus*, *Pseudomonas*, and *Rhizobium*), fungal genera (*Penicillium* and *Aspergillus*), actinomycetes, and arbuscular mycorrhizal (AM) are notable (Table 1).

Soil is a natural basal media for microbial growth. Mostly, one gram of fertile soil contains 10^1 to 10^{10} bacteria, and their live weight may exceed 2,000 kg ha^{-1} [4]. Among the whole microbial population in soil P, solubilizing bacteria comprise 1–50% and P solubilizing fungi 0.1 to 0.5% of the total respective population [4, 6, 12]. PSMs are ubiquitous, and their figures differ from soil to soil. Most PSMs were isolated from the rhizosphere of various plants, where they are known to be metabolically more active [4, 6, 23].

Apart from those species, symbiotic nitrogenous rhizobia [4, 6, 9] and nematofungus *Arthrobotrys oligospora* [4, 6, 14, 26] have also shown phosphate solubilizing activity.

4. Screening and Isolation of PSMs

Various growth mediums are being used in laboratories for isolation and characterization of PSM. The reliable approach used for preliminary screening and isolation of potential PSM was first described by Pikovskaya [28]. It works by plating 0.1 ml or 1 ml of serially diluted rhizospheric soil suspension on a sterilized Pikovaskaya's (PVK) medium supplemented with insoluble tricalcium phosphate (TCP)/ hydroxyapatite as the only P source. Colonies forming a clear halo zone around each colony are screened as PSM after incubation at appropriate temperature. Pure cultures of such colonies are further processed for identification through biochemical and molecular characterization.

P solubilizing ability of a particular PSM can be assessed in terms of the solubilization index (SI), the ratio of total diameter, i.e., clearance zone and the colony diameter. As described in several studies [8, 19, 29], phosphate SI can be determined using the following formula:

$$\mathrm{SI} = \frac{\text{colony diameter} + \text{halozone diameter}}{\text{colony diameter}}. \quad (1)$$

As described by Yousefi et al. [30], the percent of change in Pi fractions in soil in pot experiment can be determined as follows:

$$(\%)p = \left[\frac{(\mathrm{Pi}_2 - \mathrm{Pi}_1)}{\mathrm{Pi}_1}\right] \times 100, \quad (2)$$

where Pi_2 is the concentration of each fraction ($mg\cdot kg^{-1}$) in soil after cutting and Pi_1 is the concentration of each fraction ($mg\cdot kg^{-1}$) in soil before planting.

5. Mechanism of P Solubilization

PSMs apply various approaches to make phosphorus accessible for plants to absorb. These include lowering soil PH, chelation, and mineralization.

5.1. Lowering Soil pH. The principal mechanism for solubilization of soil P is lowering of soil pH by microbial production of organic acids or the release of protons [3, 5, 6, 9, 10, 18, 23, 30]. In alkaline soils, phosphate can precipitate to form calcium phosphates, including rock phosphate (fluorapatite and francolite), which are insoluble in soil. Their solubility increases with decreases in soil pH. PSMs increase P availability by producing organic acids that lowers the soil pH [5]. Strong positive correlation has been reported between solubilization index and organic acids produced [8]. PSMs are also known to create acidity by evolution of CO_2 [30], as observed in solubilization of calcium phosphates [6]. Production of organic acid coupled with the decrease of the pH by the action of microorganisms resulted in P solubilization [23]. As the soil pH increases, the divalent and trivalent forms of inorganic P, HPO_4^{-2} and HPO_4^{-3}, occur in the soil.

The PSMs may release several organic acids (Table 2). These organic acids are the products of the microbial metabolism, mostly by oxidative respiration or by

Table 1: Potential P solubilizing microorganisms.

	PSMs	Sources
Bacteria	*Bacillus circulans*	[2–5]
	Bacillus megaterium	[2–5, 12, 13]
	Bacillus polymyxa; *B. subtilis*	[2–4, 13, 14]
	Bacillus pulvifaciens	[15]
	Bacillus coagulans; *B. fusiformis*; *B. pumilus*; *B. chitinolyticus*	[2]
	Bacillus sircalmous	[3, 4]
	Thiobacillus ferrooxidans	[2]
	Pseudomonas canescens	[8]
	Pseudomonas putida	[2, 4, 14–17]
	Pseudomonas calcis	[2]
	Pseudomonas fluorescens	[2, 4, 13, 14, 17]
	Pseudomonas striata	[2–4, 13, 14]
	Pantoea agglomerans	[18]
	Rhizobium meliloti	[2]
	Rhizobium leguminosarum	[6, 13, 19]
	Mesorhizobium mediterraneum	[20]
Fungi	*Aspergillus nNiger*	[2, 8, 14, 21–24]
	Aspergillus clavatus	[8]
	Aspergillus awamori	[2, 3, 13, 14, 23, 25]
	Aspergillus candidus; *A. parasiticus*; *Aspergillus fumigatues*; *A. rugulosus*	[21, 24]
	Aspergillus flavus	[2, 14, 23]
	Aspergillus foetidus; *A. nidulans*; *A. wentii*	[2]
	Aspergillus terreus	[2, 21, 23, 24]
	Aspergillus tubingensis	[22]
	Aspergillus sydawi; *A. ochraceus*; *A. versicolor*	[23]
	Penicillium bilaii	[5, 13]
	Penicillium citrinum	[25]
	Penicillium digitatum; *P. lilacinium*; *P. balaji*; *P. funicolosum*	[2]
	Penicillium oxalicum	[14]
	Penicillium simplicissimum; *P. rubrum*	[21, 24]
	Arthrobotrys oligospora	[13, 14, 26]
	Trichoderma viride	[2, 14, 23]
Actinomycetes	*Acinetobacter rhizosphaerae*	[27]
	Streptomyces albus; *S. cyaneus*; *Streptoverticillium album*	[3]
Cyanobacteria	*Calothrix braunii*	[2]

fermentation when glucose is used as carbon source [5, 8]. The type and amount of organic acid produced differ with different organisms. Efficiency of solubilization is dependent upon the strength and nature of acids. Moreover, tri- and dicarboxylic acids are more effective as compared to monobasic and aromatic acids, and aliphatic acids are also found to be more effective in phosphate solubilization compared to phenolic, citric, and fumaric acids [6, 11]. Organic acids that solubilize phosphates are primarily citric, lactic, gluconic, 2-ketogluconic, oxalic, glyconic, acetic, malic, fumaric, succinic, tartaric, malonic, glutaric, propionic, butyric, glyoxalic, and adipic acid [3, 5, 6, 23, 30, 31]. Of these, gluconic acid and 2-ketogluconic acids appear to be the most frequent agent of mineral phosphate solubilization [5, 6, 9]. Gluconic acid is reported as the principal organic acid produced by phosphate solubilizing bacteria such as *Pseudomonas* sp. [9], *Erwinia herbicola* [9], and *Burkholderia cepacia* [9]. Another organic acid identified in strains with phosphate-solubilizing ability is 2-ketogluconic acid, which is present in *Rhizobium leguminosarum*, *Rhizobium meliloti* [9], and *Bacillus firmus* [9]. Strains of *Bacillus licheniformis* and *Bacillus amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids. It is been reported that Gram-negative bacteria are more effective at dissolving mineral phosphates than Gram-positive bacteria due to the release of diverse organic acids into the surrounding soil [3].

5.2. Chelation. Organic and inorganic acids produced by PSM dissolve the insoluble soil phosphates by chelation of cations and competing with phosphate for adsorption sites in the soil [4, 10]. The hydroxyl and carboxyl groups of the acids chelate the cations bound to phosphate, thereby converting it into soluble forms. These acids may complete for fixation sites of Al and Fe insoluble oxides, on reacting with them, stabilize them, and are called “chelates”. 2-ketogluconic acid is a powerful chelator of calcium [6].

Table 2: Diversity of organic acid produced by PSMES.

PSM isolates	Organic acids	References
Bacillus sp.	Citric acid, malic acid, succinic acid, fumaric acid, tartaric acid, gluconic acid	[23]
Pseudomonas	Citric acid, succinic acid, fumaric acid, gluconic acid, 2-ketogluconic acids	[3, 23]
Proteus sp.	Citric acid, succinic acid, fumaric acid, gluconic acid	[23]
Aspergillus	Citric acid, gluconic acid, oxalic acid, succinic acid, malic acid, glycolic acid	[29]
Azospirillum sp.	Citric acid, succinic acid, fumaric acid, gluconic acid	[23]
Penicillium sp.	Gluconic acid, glycolic acid, succinic acid, malic acid, oxalic acid, citric acid	[29]
Erwinia herbicola	Gluconic acid, 2-ketogluconic acids	[3]
Thermotolerant acetic acid	Acetobacter, Gluconobacter	[3]

Production of inorganic acids, such as sulphidric [6, 9, 32], nitric [6, 32], and carbonic acid [9], has been reported. Nitric and sulphuric acids react with calcium phosphate and convert them into soluble forms [6, 32].

5.3. Mineralization. The other mechanism of solubilizing soil P is mineralization. Organic phosphate is transformed into utilizable form by PSM through process of mineralization, and it occurs in soil at the expense of plant and animal remains, which contain a large amount of organic phosphorus compounds such as nucleic acids, phospholipids, sugar phosphates, phytic acid, polyphosphates, and phosphonates [4]. Mineralization and immobilization of soil organic P plays a vital role in phosphorus cycling of the agricultural land.

PSMs mineralize soil organic P by the production of phosphatases like phytase [1, 3–5, 21, 23, 24, 33] that hydrolyze organic forms of phosphate compounds, thereby releasing inorganic phosphorus that will be immobilized by plants. Alkaline and acid phosphatases use organic phosphate as a substrate to convert it into inorganic form. The following are among the commonly reported phytase-producing fungus: *Aspergillus candidus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus rugulosus*, *Aspergillus terreus*, *Penicillium rubrum*, *Penicillium simplicissimum*, *Pseudeurotium zonatum*, *Trichoderma harzianum*, and *Trichoderma viride* [21, 24]. Soil *Bacillus* and *Streptomyces* spp. are able to mineralize complex organic phosphates through production of extracellular enzymes like phosphoesterases, phosphodiesterases, phytases, and phospholipases [6]. Mixed cultures of PSMs (*Bacillus*, *Streptomyces*, and *Pseudomonas*) are most effective in mineralizing organic phosphate [4].

Some PSM produces siderophores, hydrolyze the organic P in the soil resulting in P availability [3–5, 23, 33].

6. Mode of Plant Growth Promotion by PSM

PSM exhibited the capacity to restore the productivity of degraded slightly productive and unproductive agricultural soils [34]. The primary means by which PSM enhance plant growth is by improving P acquisition efficiency of plants, thereby converting of the insoluble forms of P to an accessible form (orthophosphate) by plants, an essential quality of PSMs. Inoculation of PSMs in soil or seed is known to enhance solubilization of applied and fixed phosphates in soil, resulting in better crop yield [23]. It has also been reported that PSM help to absorb the phosphorus from a wider area by developing an extended network around the root system [7]. As a result, these microbial communities when employed singly or in combination with other rhizospheric microorganisms [6, 37] have shown considerable outcomes on plants in conventional agronomic soils (Table 3). Correlations between the inoculation of PSM in soil with plant height, biomass production, and phosphorus content in plants have been reported [1]. Inoculation with PSB such as *Pseudomonas*, *Bacillus*, *Rhizobium*, *Micrococcus*, *Flavobacterium*, *Achromobacter*, *Erwinia*, and *Agrobacterium* has been reported in increasing solubilization of fixed P ensuring high crop yields [5, 9].

PSMs promote plant growth via generating phytohormones, such as auxins, gibberellins, cytokinins, or polyamides [1, 25, 30, 40]. Organic acids such as carboxylic, glycolic, malonic, succinic, fumaric, and alpha-ketoglutaric acid that hasten the maturity and thereby enhance the ratio of straw as well as the total yield have also been recognized among phosphate solubilizers [6, 9, 31].

PSMs also promote plant growth indirectly by increasing the accessibility of other trace elements such as siderophore [1, 6, 9, 41]. Besides, the PSMs also facilitate plant growth by promoting the efficiency of nitrogen fixation through bio-inoculation trials [13]. Thus, production of IAA and GA coupled with phosphate solubilization by *Rhizobium leguminosarum* and *Pseudomonas* sp. (54RB) has been reported [19]. PSMs also protect plants by avoiding phytopathogens, typically owing to the production of antibiotics, hydrogen cyanate (HCN), and antifungal metabolites.

7. Trend of PSM Use as Biofertilizer and the Feature Prospect

Phosphorus use efficiency in agricultural lands can be improved through inoculation of PSM. Indications of their contribution in solubilization of inorganic phosphates and mineral phosphates were reported [21, 24, 32, 42]. Ghaderi et al. [17] demonstrated that the rate of P released by

Table 3: Effect of PSM on growth and yield performance of different crops.

PSMs	Host plant	Reference
Azotobacter	Wheat	[9]
Azotobacter chroococcum	Wheat	[36]
Azospirillum spp.	Maize, sorghum, and wheat	[9]
Bacillus	Wheat 33 (*Triticum aestivum* L.)	[6, 9]
Bacillus	Peanut, potato, sorghum, and wheat	[9]
Bacillus circulans and *Cladosporium herbarum*	Wheat	[36, 37]
Bacillus megaterium and *Azotobacter chroococcum*	Wheat	[9]
Pseudomonas	*Zea mays* L.	[6, 38]
Pseudomonas	Soybean	[6, 18]
Pseudomonas chlororaphis and *P. putida*	Soybean	[36]
Pseudomonas fluorescent	Peanut	[39]
Pseudomonas putida and *Pseudomonas fluorescens*	Canola, lettuce, and tomato	[9]
Pseudomonas putida and *Pseudomonas fluorescens*	Potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants, and wheat	[9]
Mesorhizobium mediterraneum	Chickpea and barley	[20]

Pseudomonas putida, *Pseudomonas fluorescens CHAO*, and *Tabriz Pseudomonas fluorescens* was 51, 29, and 62%, respectively. Similarly, the inoculation of *Glomus fasciculatum* and *Azotobacter* resulted in significant improvement in uptake of P, K, and N through mulberry leaf as compared to the uninoculated plants [43]. Likewise, improved phosphorus uptake and increased grain yield of wheat were reported following inoculation of phosphate solubilizing *Pseudomonas* and *Bacillus* species [6]. PSM increases the availability of P without disturbing the biochemical composition of the soil. This is essentially applicable, where access to chemical fertilizers is limited. PSM can be used for various crops and not host specific.

Several studies reported that the use of PSM enhanced growth, yield, and quality in many crops including walnut, apple, maize, rice, mustard, oil palm, aubergine and chili, soybean, wheat, sugar beet, sugarcane, chickpea, peanut and legumes, and potatoes (Table 3). PSMs have shown to enhance P uptake, the growth, and the yield when applied to crop plants [16, 40]. Adequate supply of P helps in seed formation and early maturation of crops like cereals and legumes [2]. It causes early ripening and stimulates young plants to produce deeper and abundant roots [7].

PSM improved sugarcane yield by 12.6% [44], and wheat yield up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculants [9] have been documented. Similarly, a 10–20% yield increase was reported in field trials using a combination of *Bacillus megaterium* and *Azotobacter chroococcum*. However, *Azospirillum* spp. showed increased yield in maize, sorghum, and wheat while *Bacillus* spp. revealed increased yield in peanut, potato, sorghum, and wheat [9].

Inoculation of peanut seeds with P solubilizing *Pseudomonas fluorescent* isolates significantly enhanced the nodule number and dry weight over the control [39]. Likewise, inoculation of *Pseudomonas* revealed favorable effect on slat tolerance of *Zea mays* L. under NaCl stress [38].

Yousefi et al. [30] demonstrated that phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) alone and their combination led to an increase shoot dry matter yield (both SDW and RDW), grain spike number, and grain yield of wheat. Highest shoot dry weight and root dry weight recorded were justified in terms of increment of the root and shoot length as well as phosphorus uptake by roots following PSB and AMZ application compared over the control. Also, Afzal and Bano [19] revealed that dual inoculation of Rhizobium and PSB without fertilizer (P) improved grain yield of wheat up to 20% as compared to sole P fertilizer application in pot experiment. Yet, better grain yield of wheat was observed using single and dual inoculation together with P fertilizer in grain yield of wheat.

So far, the only commercially available phosphate inoculum on a large scale is JumpStart, developed with a strain of *Penicillium bilaii* [5]. PSM's influence on cane yield and juice quality has been well established, and application of phosphorus has become an essential part of a sugarcane fertilizer programme [44]. Many PSMs are proved to be effective biofertilizers or biocontrolling agents especially *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis*, and *Pseudomonas striata* are effective biofertilizers [5].

8. Conclusion

Application of PSM by inoculating in soil appears to be an efficient way to convert the insoluble P compounds to plant-available P form, resulting in better plant growth, crop yield, and quality. *Bacillus, Pseudomonas, Rhizobium, Aspergillus, Penicillium*, and AMR are the most efficient P solubilizers for increasing bioavailability of P in soil. PSM provokes immediate plant growth by providing easily absorbable P form and production of plant growth hormones such as IAA and GA. Furthermore, PSM supports plant growth through production of siderophore and increases efficiency of nitrogen fixation. Besides, PSM acts as a biocontrol against plant pathogens via production of antibiotics, hydrogen cyanate (HCN), and antifungal metabolites. Thus, PSMs represent potential substitutes for inorganic phosphate fertilizers to meet the P demands of plants, improving yield in sustainable agriculture. Their application is an ecologically and economically sound approach. Further investigation, therefore, is crucial to explore effective biofertilizers—PSM with multiple growth-stimulating attributes at the field trial. Yet a combination of rock phosphate with PSM inoculum sounds preferable in terms of minimizing the risk of long-term total P soil deficit.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

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