

Review Article

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Phosphate Solubilizing Microbes: An Overview

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ABSTRACT

Phosphorous is one of the most abundant metallic element found in the earth's crust and present in soils in both organic and inorganic forms. Though it is present in high concentration, only 0.1% of the total P is available to plant because of poor solubility and its fixation in soil with other metallic elements in the soil such as Ca, Al, Fe to form calcium phosphate, aluminum phosphate and ferrous phosphate and thus becomes unavailable to plants. Tremendous application of chemical based P fertilizers has long term impact on the environment in terms of eutrophication, soil fertility depletion, carbon footprint this attitude compelled us to find a sustainable approach for efficient P availability in agriculture to meet the over growing demand of food. The use of efficient PSM (phosphate-solubilizing microorganisms), opens up a new horizon for better crop productivity and for greater yield performance without affecting the soil health. Phosphate solubilizing microorganism plays an important role in the plant nutrition through increase in P uptake by the plants and their use as PGPR is an important contribution to biofertilization of agricultural crops. Though some of the bacterial strain such as (*Pseudomonads* and *bacilli*) and fungal strains (*Aspergillus* and *Penicillium*) have been identified as PSM their relative performance under in situ conditions is not reliable and therefore there is a need of genetically modified strains which could lead better P solubilization and improve the plant health. The present review mostly focuses on the phosphate plant interaction, PSM, mechanism of P solubilization, PSM as plant growth promoter and genetic manipulation of several genes involved in mineral and phosphate P solubilization.

Keywords

Phosphate solubilizing microorganisms (PSM), Plant growth promoters (PGPR), Mechanism of P solubilization,

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Introduction

To meet the demand of overgrowing population it is the need of agrarian community to enhance the yield and future food supply. To overcome these problems, efforts needed to focus the soil biological system and the agro-ecosystem for better understanding the complex processes and their interactions for governing the stability of agricultural land.

The green revolution has been proved the most intellectual human activities contributing global food security and, consequently, changes the face of developing countries, such as India, from being food-deficient to having a food surplus. In present conditions there is an urgent need of second green revolution to increase the food production by around 50% in coming next 20

years to fulfill the demand of increasing population pressure (Vasil, 1998; Leisinger, 1999).

Chemical fertilizers, such as water-soluble phosphatic (WSP) fertilizers have played a significant role in the green revolution to rectify the phosphorus deficiencies. However, excessive use of chemical pesticides arises soil health issues and beyond certain limit the yield plateau get declined (Ahmed, 1995). Thus it becomes clear that conventional agricultural practices cannot sustain the production base, for too long; while, to augment crop productivity agronomists have to rely on chemical fertilizers. In this context, after nitrogen, phosphorus is an essential plant nutrient whose deficiency marked the high yield. The phosphorous is present only in micro molar or lesser quantities in the earth's crust (Ozanne, 1980) and is highly reactive with other elements in the soil.

Phosphorous is a one of the most abundant metallic elements found in the earth's crust and is present in the soils in both inorganic and organic forms (Gyaneshwar *et al.*, 2002). It is utilized or absorbed by the plants in inorganic form i.e. in orthophosphate (H_2PO_4^- and HPO_4^{2-}) (Hinsinger, 2001). It has a key role in metabolic processes such as photosynthesis, energy transfer, signal transduction, nitrogen fixation in legumes, crop quality and resistance to plant diseases are the main features associated with phosphorous nutrition (Sperber, 1958a; Khan *et al.*, 2014).

Phosphorus being a structural component of many coenzymes, phospho-proteins, phospholipids (Ozane *et al.*, 1980) also forms a part of the genetic memory "DNA" of all living organisms. It involved in transfer and storage of energy which used for growth and reproduction. Phosphorus plays a lead role in especially in photosynthesis, carbon

metabolism, and membrane formation (Wu, 2005) also the vital role in elongation of root, proliferation, and phosphorous deficiency affects root architecture (Borch *et al.*, 1999; Williamson *et al.*, 2001). A major portion of phosphorus absorbed by the plant is accumulated in grain in the form of phytic acid which becomes unavailable to plants and its deficiency negatively affects grains yield (Richardson, 1994).

Tropical and subtropical regime has acidic soil considered as extremely deficient in phosphorus with high phosphorus sorption (fixation) capacities. On average, most mineral nutrients in soil solution are present in millimolar amounts but phosphorus is present only in micromolar or lesser quantities (Ozanne, 1980). The low levels of phosphorus are due to high reactivity of soluble phosphate with other elements.

A number of heterotrophic microorganisms excreting organic acids which solubilized P that chelate cationic partners of P ions and release the P directly into solution (He *et al.*, 2002). These phosphate solubilizing bacteria (PSB) are being used as biofertilizer since 1950s. Microbial inoculants assimilate soluble P, and prevents it from adsorption or fixation (Khan and Joergesen, 2009). These microorganisms influences soil fertility through various processes viz. decomposition, mineralization and release of nutrients. Microorganisms enhance the P availability to plants through solubilization of inorganic form of P to in available form (Chen *et al.*, 2006; Kang *et al.*, 2002). Hence, microbial inoculants are used as an alternate source, which are both economic as well as eco-friendly. A continued exploration of the natural biodiversity of soil microorganisms and the optimization of microbial interactions in the rhizosphere represents a prerequisite step to develop the more efficient microbial inoculants with phosphorus-solubilizing ability.

Phosphate –Plant Interaction

Phosphorus is one the major nutrient limiting plant growth. It has diverse role in plant nutrition and promotes the development of deeper roots. The soil that is rich in phosphorus constitutes about 0.05% (w/w) phosphorus but only one tenth of this is available to plants. Most of the P (95-99%) present in the soil in the insoluble form and hence cannot be utilized by the plants due to chemical fixation in the soil and it's interaction with other metallic elements that are present in the rhizospheric area (Gaur and Gaid, 1999). To increase the availability of phosphorus for plants, large amounts of fertilizer is used on a regular basis. But the continuous application of fertilizer P is rapidly transferred to the insoluble forms (Abd Alla, 1994) and thus there is a need of phosphate solubilizing microorganism to make the P in available form to the plants.

Phosphate Solubilizing Micro-Organisms

Naturally occurring rhizospheric phosphorus solubilizing microorganism (PSM) dates back to 1903 (Khan *et al.*, 2009). Number of microbial species plays key role in P solubilization these includes bacteria, fungi, actinomycetes and even algae. Bacteria are predominant amongst them and proved more effective in phosphorus solubilization than fungi. In addition to *Pseudomonas* and *Bacillus*, other bacteria reported as P-solubilizers these are *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Phyllobacterium* etc. (Wani *et al.* 2005), *Azotobacter* (Kumar *et al.* 2001), *Xanthomonas* (De Freitas *et al.* 1997), *Enterobacter*, *Pantoea*, and *Klebsiella* (Chung *et al.* 2005). Several halophilic bacteria *Kushneria sinocarni* have also been isolated from the sediment of Daqiao saltern on the eastern coast of China, which may be useful in stress conditions; salt affected

agricultural soils (Zhu *et al.* 2011). 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) which includes *Penicillium* and *Aspergillus*, *Rhizoctonia solani*, *Trichoderma*.

Moreover, fungi in soils are able to traverse long distances more easily than bacteria and hence, may be more important to P solubilization in soils (Kucey 1983). Generally, the P-solubilizing fungi produce more acids compared to bacteria and thus leads more P-solubilizing activity (Venkateswarlu *et al.* 1984). Among the yeasts, *Yarrowia lipolytica* has potential to solubilize phosphate. Algae such as *cyanobacteria* and *mycorrhiza* have been also reported for P solubilization activity. A partial list of PSM including various groups is given in Table 1.

Mechanism of Phosphate Solubilization

The mechanism of P solubilization that is employed mostly by soil microorganisms includes: (1) release of complex compounds e.g. organic acid anions, siderophores, protons, hydroxyl ions, CO₂, (2) liberation of extracellular enzymes or it also referred as biochemical P mineralization and (3) the release of P during the degradation of substrate (McGill and Cole 1981). Thus, microorganisms have key role in the soil P cycle i.e. precipitation, sorption–desorption, and mineralization.

Inorganic phosphate solubilization

Microorganism plays an important role in P solubilization through secretion of organic acid production either by: (i) lowering the pH, or (ii) through chelation reaction of cations bound to P (iii) by competing with P for adsorption sites on the soil.

The lowering in pH of the medium suggests the secretion of organic acids by the P-solubilizing microorganisms (Whitelaw 2000; Maliha *et al.* 2004) via direct oxidation pathway that occurs on the outer face of the cytoplasmic membrane (Zaidi *et al.* 2009). When P is applied to soil it get interact with other metallic elements such as Fe, Al and Ca ions which makes the P unavailable to plants through the formation of ferrous phosphate, aluminium phosphate, calcium phosphate etc. and the release of organic acids by PSM leads the chelation reaction and because of this the bound P to other metallic elements get freed and becomes available to plants. The prominent acids that are released by PSM in the solubilization of insoluble P are gluconic acid, oxalic acid, citric acid (Kim *et al.* 1997), lactic acid, tartaric acid and aspartic acid etc. (Venkateswarlu *et al.* 1984).

The another mechanism is the production of H₂S, which react with ferric phosphate to yield ferrous sulphate with the release of phosphate (Swaby and Sperber 1958). It could be because of the activity of PSM occurs as a consequence of microbial sulphur oxidation (Rudolph 1922), nitrate production and CO₂ formation. These processes ultimately leads the formation of inorganic acids like sulphuric acid (Sperber 1958 a).

Organic Phosphate Solubilization

Phosphorus can be released in the soil from organic compounds by three groups of enzymes: (1) Nonspecific phosphatases, which leads dephosphorylation of phospho-ester or phosphoanhydride bonds in organic matter, (2) Phytases, which mostly release P which is intact in the form of phytic acid, and (3) Phosphonates and C-P Lyases, the phosphonates degrading enzyme enzymes that perform C-P cleavage in organo-phosphonates. Availability of organic phosphate compounds for plant nutrition

could be a limitation because as phosphorous is highly reactive it will interact with other metallic elements that are present in the soil in the rhizospheric area and becomes unavailable to plants which retard the plant growth and subsequently crop yield. Therefore, the capability of enzymes to perform the desired function in the rhizosphere is a crucial aspect for their effectiveness in plant nutrition (Tarafdar and Jungk, 1987; Tarafdar and Claassen, 1988).

Role of Microbial Exopolysaccharides in Phosphate Solubilization

Recently the role of polysaccharides in the microbial mediated solubilization of P was assessed by Yi *et al.* (2008). Microbial exopolysaccharides (EPSs) are polymers that mainly consist of carbohydrates excreted by some bacteria and fungi onto the outside of their cell walls. Bacterial strains such as *Enterobacter* sp. (EnHy-401), *Arthrobacter* sp. (ArHy-505), *Azotobacter* sp. (AzHy-510) and *Enterobacter* sp. (EnHy-402), has the ability to solubilize TCP (tri calcium phosphate).

Phosphate Solubilizing Bacteria as Plant Growth Promoters

There are several reports on plant growth promotion by bacteria that have the ability of solubilize inorganic and/or organic P from soil after their inoculation in soil or plant seeds (Kloepper *et al.*, 1988; Gaur and Ostwal, 1972; Subba Rao, 1982; Gerretsen, 1948; Cooper, 1959). It was reported that a strain of *Burkholderia cepacia*, commercially used as biofertilizer in Cuba which display significant mineral phosphate solubilization and moderate phosphatase activity, also improve the yield of tomato, potato, onion, banana, coffee etc. (Chabot *et al.*, 1993). Inoculation with two strains of *Rhizobium leguminosarum* selected for their P solubilization ability has been shown to

improve root colonization and growth promotion and to increase significantly the P concentration in lettuce and maize (Chabot 1996a). Also a strain of *Pseudomonas putida* stimulate the growth of roots and shoots and increased ^{32}P -labeled phosphate uptake in canola (Lifshitz *et al.*, 1987). Co-inoculation of *Pseudomonas striata* and *Bacillus polymyxa* strains showing phosphate solubilizing activity, with a strain of *Azospirillum brasilense*, resulted in significant increase in grain and dry matter yields, with a concomitant increase in N and P uptake (Alagawadi *et al.*, 1992). Several studies have shown that PSB interacts with the vesicular arbuscular mycorrhizae (VAM) by releasing phosphate ions in the soil, which causes synergistic interaction that allows for better exploitation of poorly soluble P sources (Ray *et al.*, 1981). Phylazonit-M is the commercial biofertilizer which contains the mixtures of different bacterial cultures such as *Bacillus megaterium*, *Azotobacter chroococcum* increases N and P supply to the plants (permission at No. 9961, 1992, by the Ministry of Agriculture of Hungary) similarly, other product named "KYUSEI EM", a mixed inoculums of lactic acid bacteria, the organic acid lactic acid being the agent for the mineral phosphate solubilization.

Genetic Engineering of Phosphate Solubilizing Microorganisms

Some of the genes have been reported involved in mineral and organic phosphate solubilization has been so far isolated and characterized (Rodriguez *et al.* 2006). Genetic manipulation of these genes followed by their expression in selected rhizobacterial strains leads a promising perspective for obtaining PSM strains with higher phosphate solubilizing capacity, and thus it become more effective as agricultural inoculants. The

initial achievement was achieved by Goldstein and Liu (1987) from the Gram negative bacteria *Erwinia herbicola* through cloning of P solubilization genes. Similarly, the napA phosphatase gene from the soil bacterium *Morganella morganii* was transferred to *Burkholderia cepacia* IS-16, a strain used as a biofertilizer, using the broad-host range vector pRK293 (Fraga *et al.*, 2001). Introduction of P solubilization genes in natural rhizosphere bacteria is a candidate approach for the improvement of microbial capacity.

Fourteen different nonspecific acid phosphatase encoding genes have been isolated from different bacterial species using different expression cloning system (Rossoloni *et al.*, 1984). Sequence analysis of the cloned phosphatase genes allowed the classification into three different families: class A, class B, and class C phosphatases (Thaller *et al.*, 1994; Thaller *et al.*, 1995a; Thaller *et al.*, 1997). Several other phosphatase genes have been isolated from *Escherichia coli*. These include: ushA, which encodes a 5'-nucleotidase (Burns *et al.*, 1986); agp, which encodes an acid glucose-1-phosphatase (Pradel *et al.*, 1990) and cpdB, encoding the 2'-3' cyclic phosphodiesterase (Beacham *et al.*, 1980). Despite the difficulties, significant progress has been made for obtaining genetically engineered microorganisms for the agricultural use (Armarger 2002).

In conclusion, phosphorus is an essential element in crop nutrition. Continuous application of chemical based P fertilizers compelled us to find a sustainable approach for efficient P availability in agriculture to meet the over growing demand of food. Soil microorganisms plays varied role that affect the transformation of P and thus influence the availability of P to plant roots.

Table.1 Biodiversity of P Solubilizing Microorganisms

Groups	Examples
N₂ fixing biofertilizers	
Free living	<i>Azotobacter, Beijerinckia, Clostridium, Klebsiella, Anabaena</i> and <i>Nostoc</i>
Symbiotic	<i>Rhizobium, Frankia</i> and <i>Anabaena azollae</i>
Associative symbiotic	<i>Azospirillum</i>
P solubilising biofertilizers	
Bacteria	<i>Bacillus megaterium</i> var. <i>phosphaticum</i> , <i>Bacillus subtilis</i> , <i>Bacillus circulans</i> and <i>Pseudomonas striata</i>
Fungi	<i>Penicillium</i> sp. and <i>Aspergillus awamori</i>
P mobilizing biofertilizers	
Arbuscular mycorrhiza	<i>Glomus</i> sp., <i>Gigaspora</i> sp., <i>Acaulospora</i> sp., <i>Scutellospora</i> sp. and <i>Sclerocystis</i> sp.
Ectomycorrhiza	<i>Laccaria</i> sp., <i>Pisolithus</i> sp., <i>Boletus</i> sp. and <i>Amanita</i> sp.
Ericoidmycorrhiza	<i>Pezizella</i>
Orchid mycorrhiza	<i>Rhizoctonia solani</i>
Biofertilizers for micro nutrients	
Silicate and Zinc solubilizers	<i>Bacillus</i> sp.
Plant growth promoting rhizobacteria	
<i>Pseudomonas</i>	<i>Pseudomonas fluorescens</i>

The use of efficient PSM (phosphate-solubilizing microorganisms), opens up a new horizon for better crop productivity and for greater yield performance without affecting the soil health. Phosphate solubilizing bacteria play an important role in the plant nutrition through increase in P uptake by the plants and their use as PGPR is an important contribution to biofertilization of agricultural crops. Therefore, steps should be taken for extensive and consistent research for the identification and characterization of PSM with greater efficiency for their ultimate application under field conditions. It becomes the responsibility of soil scientists and microbiologists how soil P could be improved without applying the chemical based phosphatic fertilizers under different agro-climatic regions.

Genetic engineering of the phosphate solubilizing character must eventually be directed to the chromosomal integration of the gene for higher stability of the character and to avoid horizontal transfer of the inserted gene in soil. This strategy would also prevent the risk of metabolic load caused by the presence of the plasmid in the bacterial cell.

Thus the exploitation of the candidate soil microorganisms which play an important role in the mobilization of soil P and understanding the mechanism of phosphate solubilization, phosphate plant interaction and their contribution to the cycling of P in soil-plant systems is essential for the development of sustainable agriculture to forward and accomplished our movement from a green revolution to an evergreen revolution.

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