

Phosphate solubilizing microbes: A sustainable tool for healthy agriculture

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ABSTRACT

Phosphorous is second most essential element after nitrogen which is required by plants for their growth and development. Plant requires phosphorous for molecules like ATP, nucleic acids, and phospholipids but its availability is 0.2% of plant dry weight. Phosphorous is present in soil in organic and inorganic form but it isn't available for plants as it is in insoluble form. A sufficient amount of phosphorous for plant during early stages of development is important which lay down the primordial of plant reproductive parts. To satisfy phosphorus requirements farmers uses chemical fertilizers, however synthesis of these chemical fertilizers requires high energy input, and it also has long term impact on environment in view of eutrophication, carbon footprint and soil fertility depletion. These environment concerns had led to search of environment friendly or sustainable mode of phosphorus uptake. In this regard phosphate solubilizing microorganism has been reported as best option for P nutrition uptake of plants. This review focuses on these phosphate solubilizing microorganism (PSM), their mechanism on how they help in uptake of phosphorous, impact of phosphate solubilization on plant growth and future scenario of their use to manage a sustainable environmental system.

Key words : Biofertilizers, Chemical fertilizers, Phosphate solubilizing microorganisms, Plant growth promoting rhizobacteria, Phosphorous

Introduction

The importance of Phosphorus cannot be overstated especially when it belongs to the class of macronutrients essential for optimum plant growth. Even though it only makes 0.2% to 08% of plants dry weight, its involvement in key important molecules such as ATP, nucleic acid, and phospholipids, makes it even more vital in plant growth and development (Kalayu, 2019). Without a reliable supply of P, plants cannot grow (Zhu *et al.*, 2011; Razaq *et al.*, 2017). It is involved in several key plant functions such as photosynthesis, transformation of sugar and starch, energy transfer, and transfer of genetic infor-

mation from one generation to another. It also plays an important role in cellular processes such as maintenance of membrane structures, synthesis of biomolecules, formation of high-energy molecules, cell division, enzyme activation/inactivation, and carbohydrate metabolism (Razaq *et al.*, 2017).

Phosphorus is considered to be growth limiting macronutrient due to its low availability in soil (Santana *et al.*, 2016). This often leads the deficiency and limiting the growth of crops. Diagnosing its deficiency is a tedious task since crops generally display no visual symptoms at an early stage. Its deficiency is often confused with nitrogen since the veins of young leaves appear red under both defi-

ciencies (Santana *et al.*, 2016).

To compensate for the low P availability in the soil, chemical P fertilizers are used but the soil is not able to hold the added P. The phosphorus use efficiency (PUE) for these fertilizers is 15-20% (Smith *et al.*, 2003; Malhotra *et al.*, 2018), which indicates that most of the applied phosphorus remain unavailable because 75-90% added chemical P fertilizer becomes fixed in the soil as it gets precipitated by metal-cation complexes. This fixed phosphorus poses a serious environmental hazard and often leaches into ground and surface water causing eutrophication and soil depletion (Kalayu, 2019).

The use of microorganisms in agriculture as biofertilizers has been a subject of study for many years. Microorganisms play an important part in the inorganic phosphorus cycle (Kalayu, 2019). Phosphate solubilizing microorganisms (PSM) through various mechanisms of solubilization can convert organic and inorganic phosphorus in soil respectively (Sharma *et al.*, 2013). This review is intended to provide a brief on the availability of phosphorus in soil, PSM diversity and mechanisms of phosphorus solubilization, benefits of PSM as biofertilizers in crop production, and future aspects of PSM-based biofertilizer.

Phosphorus content in the soil

The average content of phosphorus in soil is about 0.05% (w/w). This low availability is due high reactivity and the fixation of phosphorus in the form of aluminum/iron or calcium/magnesium phosphates (Zhu *et al.*, 2011). This insoluble, precipitated phosphorus cannot be absorbed by plants and only 0.1% of that is usable (Zhu *et al.*, 2011). The phosphorus fixation is governed by two types of reaction a) phosphate sorption on the surface of the soil and b) Precipitation of phosphate by iron and aluminium ion in the soil solution (Sharma *et al.*, 2013; Havlin *et al.*, 1999).

Plant cell uptakes phosphorus in several forms, but primarily it is absorbed in the form of orthophosphates such as H_2PO_4^- and HPO_4^{2-} depending on soil pH (Kalayu, 2019; Satyaprakash *et al.*, 2017). The chemical fertilizer used to supplement phosphorus in soil contains phosphate anions that are extremely reactive to Ca^{2+} , Fe^{3+} , and Al^{3+} ions and become fixed through interactions in the soil. This form insoluble phosphate complexes such as calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) (in alkaline soil), aluminum phosphate (AlPO_4), and ferrous phosphate

($\text{Fe}_3(\text{PO})_4$) (in acidic soil) respectively (Chen and Liu, 2019; Satyaprakash *et al.*, 2017; Walpola and Yoon, 2012). These complexes are insoluble and hence cannot be used by plants. These accumulated phosphates can remain for 100 years in the soil and is sufficient to sustain and support maximum crop yields worldwide (Walpola and Yoon, 2012; Kalayu, 2019). PSM through various mechanisms can solubilize and convert this accumulated phosphorus for plant use.

Heterogeneity of PSM in soil

The soil contains a lot of microorganisms among which phosphate solubilizing microorganisms are present in huge quantity. The microorganisms like *Rhizobium*, *Pseudomonas*, *Bacillus* (David *et al.*, 2014), *Fusarium*, *Penicillium*, *Aspergillus* are having the capability of solubilizing phosphate efficiently (Whitelaw *et al.*, 2000). There are many strains of bacteria which are considered as fundamental strains like *Bacillus circulans*, *Enterobacter*, *Pseudomonas striata* and *Bacillus subtilis* (Subbarao *et al.*, 1998). Phosphate solubilizing microorganisms contribute to the 50% of the microbial populace present in the soil (Walpola *et al.*, 2012). Normally there are more bacilli in soil while spirilli are extremely uncommon in natural habitats (Baudoin *et al.*, 2002). The availability of phosphate Solubilizing microorganism in various forms in a particular region depends upon the characteristics of soil in that region (Kim *et al.*, 1998).

Occurrence of Phosphate Solubilizing bacteria

The rhizospheric soil is found to have more microbial density among which phosphate solubilizing microorganisms are present in large quantity and they are found to be more actively involved in metabolic processes (Vazquez *et al.*, 2000). The rhizosphere is characterized as the soil around the roots that is impacted by the root. Due to the arrival of effectively decomposable mixtures by the roots (root exudates), the rhizosphere is portrayed by high microbial density. The Phosphate solubilizing bacteria which are present in rhizospheric region are found to be more metabolically active (Selvi *et al.*, 2017). Usually, one gram of rich soil contains 101 to 1010 organisms, soil microorganisms are in cocci (circle, 0.5 μm), bacilli (bar, 0.5–0.3 μm) or spiral (1-100 μm) shapes (1-100 μm) shapes (Sharma *et al.*, 2005). Bacilli are typical in soil, while spirilli are incredibly phenomenal in native natural surround-

ings. Greater masses of PSB are found in rural and rangeland soils. Plants enlist explicit microbial networks from the soil through root exudates, which contrast in arrangement across plant species (Richardson *et al.*, 2020).

Phosphate Solubilizing Bacteria

Unlike Phosphate Solubilizing bacteria, Phosphate Solubilizing Fungi is having less capability of solubilizing phosphate (Chen, 2006; Alam *et al.*, 2002). Microorganisms engaged with phosphorus obtaining incorporate mycorrhizal organisms and PSMs (Fankem *et al.*, 2006). There are many bacterial strains present in soil which are having high phosphate solubilizing capability like *Pseudomonas* and *Bacilli* (Igal *et al.*, 2001).

Benefits of Phosphorous Solubilizing Bacteria

Phosphate solubilizing bacteria can be used as biofertilizer, to convert phosphate into soluble forms to make it easily available (Zhun *et al.*, 2012). It is noted that use of PSM in agriculture can increase food availability as it is having capability of increasing agricultural yield and increases soil fertility, moreover it is harmless to the ecosystem approach (Babalola and Glick, 2012). PSM fill in as biofertilizers as it protects plants against various pathogens and also stimulates plant growth, helps in fixing atmospheric nitrogen (Wani *et al.*, 2007).

It is noted that growth of plants is boosted by the phosphate solubilizing bacteria (Tallapragada and Gudimi, 2011). PSB protect crops against many pathogens as it plays a significant role as biological control agents. Phosphate solubilizing bacteria hinder the growth of pathogens as it produces antimicrobial compounds, various antibiotics, siderophores due to which they promote the development of crops.

PSB replaces the chemical fertilizers as it does not cause any harm to soil or environment moreover enrich soil fertility and increases the crop yield. It is less expensive as compared to chemical fertilizer and also does not cause any harm to microbial population. The solubilization of both inorganic and organo-phosphorous is found to be done by *Kushneria sp.* YCWA18 (Zhu *et al.*, 2011). During variable NaCl concentrations, the solubilization of tricalcium phosphate is found to be done by microorganisms like *Aspergillus sp.* Strain, *Pseudomonas aeruginosa* strain (Srinivasan *et al.*, 2012). It is found that during variable NaCl concentrations the devel-

opment and growth of maize plant is promoted by *Bulkholderia cepacia* (Zhao *et al.*, 2012). It is considered that PSB also plays a significant role as a biofertilizer in promoting growth of crops in various alkaline conditions.

PSM Mechanism for Phosphate solubilisation

PSM apply major processes to make the phosphorous available to the plants for absorption. The soil Phosphate concentration is affected by soil P cycle processes such as 1) dissolution and precipitation 2) sorption and desorption and 3) mineralization and immobilization (Sims and Pierzynski, 2005).

Inorganic Phosphate Solubilisation

Phosphorous present in the soil is bound to Iron, Aluminium and Calcium. These are solubilised by following ways.

Production of Organic Acids

PSMs produce organic acids such as Citric acid, tartaric acid, gluconic acid, oxalic acid that solubilise inorganic phosphates by 1) Chelation 2) lowering pH 3) complexation with metal ions bound to phosphates and 4) challenging P for adsorption site (Kishore *et al.*, 2015). The Phosphorous in the soil interacts with elements such as Fe, Al and Ca ions forming ferrous phosphate, aluminium phosphate, calcium phosphate. This makes the Phosphorous unavailable to plants. PSM releases organic acids which leads the chelation reaction resulting in the release of bound Phosphorous from metallic elements making it available to plants (Bashir *et al.*, 2017). Organic acids produced with their carboxyl and hydroxyl ions reduce the pH in the soil thereby releasing Phosphorous (Seshachala and Tallapragada, 2012); The organic acids are produced in the periplasmic space of Phosphate Solubilising bacteria by the mechanism called direct oxidation pathway (Zhao *et al.*, 2014). Gluconic acid is the most frequent agent of mineral phosphate solubilisation. This acid is useful as it chelates the cations bound to phosphate thereby making the phosphorous available. Using the mechanism called direct oxidation pathway Gram-negative bacteria solubilize mineral phosphate by converting glucose to gluconic acid (Goldstein, 2000).

Production of Inorganic acids

Inorganic acids produced by PSMs also solubilise phosphate. Inorganic acids produced has low effi-

ciency rate to solubilise the phosphate in the soil compared to organic acids. *Nitrobacter* and *Thiobacillus* spp., produces nitric acid and sulfuric acid respectively which helps to dissolve Phosphorous (Shrivastava *et al.*, 2018). In 1997, it was reported that HCl solubilise Phosphate by lowering the pH.

Production of H₂S

H₂S is produced as a metabolic byproduct by Acidophilic and sulfur-oxidizing bacteria which reacts with ferric phosphates and forms ferrous sulphate releasing the bound phosphorous (Florentino *et al.*, 2016). The production of Hydrogen Sulphide makes it react with ferric phosphate. This yields ferrous sulphate releasing phosphate. It could be the activity of Phosphate solubilising microorganisms which are microbial sulphur oxidation, nitrate production and CO₂ formation, producing inorganic acids like sulphuric acid (Padole *et al.*, 2017).

Ammonium releasing Proton

PSMs assimilate Ammonium for the production of Amino acids. Inside the microbial cell, Ammonium is converted to Ammonia releasing the excess protons into the cytoplasm acidifying the medium. Thus the acidified medium of the microbial cell helps in solubilisation of insoluble phosphates (Gand, 2016). It was reported that when Ammonium was used as a nitrogen source instead of Nitrate, the amount of Phosphate dissolved was high (Sharan and Darmwal, 2008). When media was supplemented with Ammonium as nitrogen source, *Bacillus subtilis* BPM12 strain could solubilise maximum phosphorous (Wang *et al.*, 2020).

Indirect mechanisms

Direct Oxidation Pathway

In this pathway glucose is converted to gluconic acid by enzyme glucose dehydrogenase. It is further oxidised to 2-ketogluconic acid by enzyme gluconate dehydrogenase. Gluconic acid and 2-ketogluconic acids produced in the direct oxidation pathway act as chelators of minerals like calcium and ferrous iron from their phosphate bound form (Krishnaraj and Goldstein, 2001). *Pseudomonas aeruginosa* KR270346 also solubilises phosphorous by secreting gluconic acid which is a dominant acid by direct oxidation pathway (Linu *et al.*, 2019). Pyrroloquinoline quinone (PQQ) is both a redox

cofactor and antioxidant. This PQQ act as a redox cofactor in glucose dehydrogenases (GDH) aiding in phosphate solubilisation (Rodríguez *et al.*, 2000).

Siderophore Production

Siderophore are low molecular weight with high-affinity for iron-chelating compounds and strongest ferric ion complexing agents (Birch and Bachofen, 1990). PSMs release siderophores to chelate iron from Ferrous Phosphorous complexes in the soil (Collavino *et al.*, 2010). Novel fungi *Beauveria brongniatii* secreted siderophores resulting in about 59.8% of Ferric iron-Chrome azurol_S degradation. Then solubilised 158.95 mg/L phosphorous *in vitro* (Toscano-Verduzco *et al.*, 2020). When *Enterobacter* spp. and *E.coli* was plated on CAS agar, the production of siderophore was observed. A colour change was qualitatively observed from blue to purplish red around the colonies of both bacteria (Gustavo *et al.*, 2020).

Exopolysaccharide Production

Exopolysaccharides can be either homopolymers or heteropolymers of carbohydrates (Sutherland, 2001). Exopolysaccharide production by soil microflora is also enhanced under stress conditions (Silambarasan *et al.*, 2019). In the strain of *Paenibacillus polymyxa* GOL 0202, exopolysaccharide production was found along with phosphate solubilisation effect (Cherchali *et al.*, 2019).

Organic Phosphate Solubilization

Organic Phosphorous constitute 20-30% of the total phosphorous in the soil and its dissolution occurs by action of enzymes (Kumar and Shastri, 2017). NSAPs are also known as phosphomonoesterases and are of two types: acid and alkaline phosphatases that are secreted by PSMs (Nannipieri *et al.*, 2011). NSAPs are also known as phosphomonoesterases and are of two types: acid and alkaline phosphatases that are secreted by PSMs (Nannipieri *et al.*, 2011).

Non-Specific Acid Phosphatases (NSAPs)

The non-specific acid phosphatases (NSAPs) is an enzyme that dephosphorylate the phospho-ester or phosphoanhydride bond of organic compounds (Nannipieri *et al.*, 2011). NSAPs are known as phosphomonoesterases. Phosphomonoesterases produced by PSM are of 2 types, acid phosphatases and alkaline phosphatases (Nannipieri *et al.*, 2011). Alka-

line phosphatases hydrolyze about 90% of organic phosphorous in soil (Jarosch *et al.*, 2015). When purified alkaline phosphatases from *Bacillus licheniformis* MTCC 2312 was inoculated in soil, it improved the phosphorous content in the root and stem of *Zea mays* L (Singh and Banik, 2019).

Phytases

This enzyme catalyses the removal of phosphorous from the phytate compound (Sharma *et al.*, 2013). The presence of PSM within the rhizosphere makes the Phosphorous available to the plants since they cannot acquire it from phytate (Richardson and Simpson, 2011). Elite strains of *Pseudomonas corrugate* SP77 and *Serratia liquefaciens* LR88 displayed phytase activity up to 23.02 and 24.84 U m/l, respectively (Ben Zineb *et al.*, 2020).

Phosphonatases and C-P Lyases

This enzyme catalyses the cleavage of Carbon Phosphorous (C-P) bond of organophosphates making the phosphorous available (Rodriguez *et al.*, 2006). Carbon Phosphorous (CP) lyases activity is reported in many phosphate solubilising bacteria. Some of these are *Bacillus*, *Pseudomonas*, *Enterobacter*, *Acinetobacter*, *Rhizobium* and *Burkholderia* (Teng *et al.*, 2019; Vazquez *et al.*, 2020). CP lyases activity is also reported in endophytic fungi such as *Aspergillus*, *Penicillium*, *Piriformospora* and *Curvularia* (Mehta *et al.*, 2019).

Plant growth promoting PSM

PSM possess the property of restoring productivity of unproductive or less productive soil (Gyaneshwar *et al.*, 2002). Plant growth is enhanced by improving P additive efficiency of plants by PSM. An essential quality of PSMs is to convert the insoluble form of phosphate to a form which is acceptable by plants. Fixed phosphate solubilisation enhancement is known to be done by inoculation of PSMs in soil or seed which results in better crop yield (Selvi *et al.*, 2017). It has been reported that PSM helps the root system in absorbing the phosphorous from a wider area of soil by developing an extended network around the root system (Mehrvarz *et al.*, 2008). As a result of it these microbial communities have shown considerable outcomes on plants when given singly (one species type) or in combination with other rhizospheric microorganisms in conventional agronomic soils (Walpola *et al.*, 2012).

Some relations when inoculation of PSMs in soil is done with production of biomass, plant height and phosphorous content in plants have been seen (Santana *et al.*, 2016). PSMs promote plant growth by generating phytohormones, such as gibberellins, auxins, polyamides or cytokinins (Santana *et al.*, 2016). Some PSB like *Pseudomonas*, *Rhizobium*, *Micrococcus*, *Bacillus*, *Achromobacter*, *Erwinia*, *Flavobacterium*, and *Agrobacterium* have been noticed in enhancing the solubility of fixed P to ensure the increment in crop yield (Kumar *et al.*, 2014; Satyaprakash *et al.*, 2017). Organic acids such as glycolic, carboxylic, succinic, fumaric, malonic and alpha-ketoglutaric acid have been recorded among phosphate solubilizers which increase the maturity and enhance the ration of straw and the total yield.

PSMs increase the accessibility of other trace elements such as siderophore which results in indirect promotion of plant growth. PSM also helps in increasing the efficiency of N fixation by bioinoculation trials method (Hajjam *et al.*, 2017). Production of gibberellic acid (GA) and indole acetic acid (IAA) entangled with P solubilizers such as *Pseudomonas sp.* (54RB) and *Rhizobium leguminosarum* have been reported (Kumar *et al.*, 2018). PSMs also produce antibiotics, hydrogen cyanate (HCN), and antifungal metabolites to protect plants by avoiding phytopathogens.

Future aspects

The research shows that the inoculation of phosphate solubilizing bacteria is a better alternative of phosphate biofertilizers because of its organic nature and high efficiency. Future research should be directed towards lowering the dependability of plants on phosphate biofertilizers and making the crop production more economic and environmentally safe.

More research work should be focused on studying the biochemical nature and molecular mechanism of the phosphate solubilizing bacteria in rhizospheric soil. A detailed study is required on the synergistic interactions of phosphate solubilizing bacteria with other rhizospheric microbes.

A separate research should be done based on the genetic modifications of the phosphate solubilizing bacteria and other rhizospheric -competent microbe which could be enhanced genetically to perform as phosphate solubilizing bacteria for the plants to increase the efficiency and fertility of the soil and its phosphate solubilization mechanism. Stability and

performance of phosphate solubilizing Bacteria and genetically modified PSM requires a complete study. The future depends on the stability and performance of those PSM.

Conclusion

This review has shown that phosphate solubilizing microorganisms are a better alternative for plants and sustainable agriculture. Phosphate is one of the most important chemical components in plant growth and crop production. The use of phosphate solubilizing microbes increases crop productivity as well as assure environment safety by mobilizing inorganic phosphate and making it available to harness by plants. The use of PSM enhances the soil fertility which could be used for longer period. However, the field required few developments to study the genetic modifications of the PSMs in rhizospheric soil.

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Conflict of Interest

Authors declare no conflict of interest.

Authors' Contribution

All authors have direct and intellectual contribution to the work, and approved it for publication.

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