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REVIEW ARTICLE

AN ECO-FRIENDLY APPROACH FOR MANAGING PHOSPHORUS DEFICIENCY IN AGRICULTURAL SOILS: BY USING PHOSPHATE SOLUBILIZING MICROBE

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ABSTRACT

Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of chemical P fertilizer, however the synthesis of chemical P fertilizer is a highly energy intensive processes, and has long term implications on the environment in terms of eutrophication, soil fertility depletion, destruction of micro-organisms and carbon footprint. Plants can use only a small amount of applied P, since 75–90% of added P is precipitated by metal–cation complexes, and rapidly becomes fixed in soils. Such environmental concerns have led to the search for sustainable way to provide P nutrition to crops. In this regards, phosphate solubilizing microorganism have been seen as best ecofriendly tools to generate solubilized P for plants, provide resistance against plant pathogens and stimulating the production of essential phytohormones. However, their performance under in situ conditions is not reliable and therefore needs to be improved by using either genetically modified strains or co-inoculation techniques. In recent years, several phosphatases encoding genes have been cloned and characterized which prove to be the excellent for plant growth. Therefore, genetic modification of phosphate solubilizing bacteria includes gene cloning, followed by their expression in selected rhizobacterial strains is an interesting approach. Besides phosphate solubilizing activity of microorganisms, this review article focuses on diversity of PSM, mechanism behind P solubilization, genes involved in phosphate solubilization, co-inoculation of PSB with other microbes. In addition, it also describes possible future scenario of their use.

Highlights

- Described phosphorus deficiency in agricultural soils and its management by using phosphate solubilizing microorganisms.
- Future scenario of their use and potential for application of this knowledge in managing a sustainable agro-ecological system.

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INTRODUCTION

Phosphorus (P) is a component of the nucleic acid structure which regulates protein synthesis and plays an important role in biological growth and development in plants. Being the most limiting macronutrient after nitrogen, P plays a vital role in biochemical pathways, cell division, photosynthesis and development of new tissue (Richardson *et al*., 2011). Along with these essential functions, P is also associated with complex signal transduction, macromolecular biosynthesis, energy transformations, respiration, increasing root ramification and strength as well as provides disease resistance in the plant (Khan *et al*., 2010). Soil P exists in various chemical forms including inorganic P (Pi) and organic P (Po); both differing in their behavior and fate in soil (Hansen *et al*., 2004; Turner *et al*., 2007). Pi accounts for 35% to 70% of total P in soil, while Po generally accounts for 30% to 65%. The inorganic portion occurs in various combinations with iron, aluminum, calcium, and other elements. However, organic P (Po) in soil is largely in the form of hexaphosphate, phospholipids, nucleic acids, inositol phosphates and phytates. Since P is a stable element in soils, it does not form a gas (like ammonia), therefore cannot move far from where it is applied

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and becomes unavailable to plants. The reason for the stability of phosphate compounds in the soil is that they are highly reactive and react rapidly with other compounds. To overcome this problem, most of the farmers regularly use chemical phosphate fertilizers which get incorporated into the soil. These applied P easily transforms into an insoluble and stable form with limited availability to plants and only 5% or less of the total amount of P in soil is available for plant nutrition. The phenomena of fixation and precipitation of P in soil is highly dependent on pH and soil type. Thus, in acid soils, phosphorus is fixed by free oxides and hydroxides of aluminum and iron, while in alkaline soils it is fixed by calcium, causing a low efficiency of soluble P fertilizers, such as super calcium. Given the negative environmental impacts of chemical fertilizers and their increasing costs, the use of phosphate solubilizing microorganisms (PSM) is advantageous in the sustainable agricultural practices. The use of microbial inoculants (biofertilizers) possessing P-solubilizing activities in agricultural soils is considered as an environmental-friendly way which is an alternative to applications of chemical based P fertilizers. Microorganisms use several direct and indirect mechanisms of action to improve plant growth and health by suppression of plant disease (through the production of antibiotics, phenazines and antifungal metabolites, etc.), phytohormone production (Biostimulants), N_2 fixation, siderophore production, regulation of plant ethylene levels through the ACC-deaminase enzyme and response to external stress factors. Several species of *Azospirillum* are able to secrete phytohormones such as auxins, gibberellins, cytokinins, and nitric oxide, which are directly involved in plant growth and productivity (Fibach-Paldi *et al*., 2012; Rojas *et al*., 2001; Gergonius *et al*., 2015).

The involvement of microorganisms in solubilization of inorganic phosphates was known as early as 1903 (Khan *et al*., 2007). Since then, there have been extensive studies on phosphate solubilization by naturally abundant rhizospheric microorganisms. Important genera of phosphate-solubilizing microorganism (PSM) include *Alcaligenes sp., Aerobactor aerogenes, Achromobacter sp., Actinomadura oligospora, Pseudomonas sp., Bacillus sp., Burkholderia sp. and Rhizobium sp*. Apart from bacterial species some fungal stains are also reported as phosphate solubilizers such as *Aspergillus sp.* (*Aspergillus awamori, A. niger, A. tereus, A. flavus, A. nidulans, A. foetidus, A. wentii.)* and *Penicillium sp.* (*Penicillium digitatum, P lilacinium, P balaji, P. funicolosum).* Population of these microorganisms also depends on different agriculture soil properties (physical and chemical properties, organic matter, and P content), cultural activities, frequency and percentage of occurrence in soil. However, PSM are more suitable for high-volume production of crop but their numbers are not high enough in the rhizosphere. Therefore, crop yield improvement requires inoculation of target microorganism at a much higher concentration. The diversity and richness of established PSM varies from soil to soil depending upon the physicochemical properties. P solubilizing ability of PSM also varies from organism to organism and even in different strains of the same species. Thus, their biodiversity assessment along with soil physicochemical variables and screening of better strains is necessary for developing a soil specific biofertilizers. This review is an effort to emphasize how the beneficial association between plants and PSM can be used to remediate the P deficient soils and to address the developmental status of the current trend in application of PSM in this context.

Biodiversity of P solubilizes

The last two decades have seen a significantly increased knowledge on phosphate solubilizing microorganisms (PSM) (Richardson, 2001). Several strains of bacterial and fungal and some Actinomycetes species have been identified and investigated in detail for their phosphate-solubilizing capabilities. The PSM is present in all the soil types and their population is higher in the rhizosphere as compared to the nonrhizosphere. They are an important component of soil and directly or indirectly influence the soil's health through their beneficial or detrimental activities by releasing P from inorganic and organic pools of total soil P through solubilization and mineralization process. Both the solubilization and mineralization of phosphate is attributed to the production and release of organic acids and acid phosphatases, respectively (Park *et al*., 2009). The phosphate solubilizing ability is greatly affected by various environmental parameters such as temperature, pH, carbon and nitrogen sources (Mujahid *et al*., 2015). Also, diversity in the PSM community is impacted by physicochemical parameters of different soil samples such as high concentrations of sodium, alkaline pH and high electrical conductivity (Mohan *et al*., 2015). These microorganisms are generally isolated from rhizosphere and nonrhizosphere soils, rhizoplane, phyllosphere, rock P deposit area soil and even from stressed soils using serial plate dilution method or by enrichment culture technique (Zaidi *et al*., 2009).

Biodiversity of Phosphate Solubilizing Bacteria (PSB)

Among the whole microbial population in soil, phosphate solubilizing bacteria (PSB) constitute 1–50% in P solubilization potential. The concentration of PSB in soil is higher in comparison with non-rhizosphere soil. The bacteria such as *Alcaligenes sp., Aerobactor aerogenes, Achromobacter sp., Actinomadura oligospora, Pseudomonas, Bacillus, Burkholderia* and *Rhizobium* are the most important phosphate solubilizers in soils. In addition, bacteria of the genus *Rhizobium* (i.e *Rhizobium leguminosarum*) play a very important role in agriculture by inducing nitrogen-fixing nodules on the roots of legumes (such as peas, beans, clover and alfalfa) and export the fixed nitrogen to the host plants, have also shown phosphate solubilizing activity (Zaidi *et al*., 2009; Abril *et al*., 2007; Sridevi *et al*., 2007). The other bacteria reported as phosphate solubilizers include *Rhodococcus, Arthrobacter, Serratia, Chryseobacterium, Gordonia, Phyllobacterium, Delftia sp*. (Chen *et al*., 2006), *Azotobacter, Pantoea and Klebsiella* (*Chung et al*., 2005). Furthermore, symbiotic nitrogenous rhizobia, which fix atmospheric nitrogen into ammonia and export the fixed nitrogen to the host plants, have also shown phosphate solubilization activity. For instance, *Rhizobium leguminosarum* bv. *trifolii* (*Abril et al*., 2007) *R. leguminosarum* bv. *Viciae* (Alikhani *et al*., 2007) and *Rhizobium* species nodulating *Crotalaria* species (Sridevi M *et al*., 2007) improved solubilize phosphates by mobilizing inorganic and organic P.

Biodiversity of Phosphate Solubilizing Fungi (PSF)

Apart from bacterial sp. some fungal stains are also reported as phosphate solubilizers such as *Aspergillus sp. (Aspergillus awamori, A. niger, A. tereus, A. flavus, A. nidulans, A. foetidus, A. wentii.* and *Penicillium sp. (Penicillium digitatum, P lilacinium, P balaji, P. funicolosum).* In soil, P-solubilizing fungi constitute about 0.1–0.5% of total fungal populations.

Table 1. Biodiversity of PSM

	Microorganisms	Reference
Bacteria	*Acinetobacter sp., Acinetobacter calcoaceticus*	Peix *et al*., 2009.
	Alcaligenes sp., Alcaligenes faecalis	Muhammad Shahid *et al*., 2015,
	Bacillus sp., Bacillus circulans, B.cereus, B.fusiformis, B. pumils, B. megaterium, B. mycoides, B. polymyxa, B. coagulans B,.chitinolyticus, B. subtilis	Nandini, K *et al*., 2014 Wani *et al*., 2007
	Enterobacter sp., Enterobacter intermedium, E. aerogenes E. tayloraer, E. asburiae	Lopez *et al*., 2011
	Pseudomonas sp., P putida, P. striata, P. fluorescens, P. calcis,	Hoon *et al*., 2003
	Rhizobium sp., Rhizobium meliloti Rhizobium leguminosarum Rhizobium loti, Mycobacterium sp.	
Fungi	*Aspergillus sp., Aspergillus awamori, A. niger, A. tereus, A. flavus, A. nidulans, A. foetidus, A. wentii, A tubingensis*	Rashmi Awasthi *et al*., 2011
	Penicillium sp., Penicillium rugulosum, P digitatum, P lilacinium, P balaji, P. funicolosum, P expansum.	
Actinomycetes	*Actinomyces, Streptomyces.*	Maloy Kumar Sahu *et al*., 2000
Cyanobacteria	*Nostoc sp.*	Singh S. *et al*., 2011
VAM	*Glomus fasciculatum.*	Almas Zaidi *et al*., 2006

Table 2. Organic acids production by phosphate solubilizing microorganisms

Bacterial and fungal strains	Organic acids	References
Pseudomonas sp., Erwinia herbicola, Burkholderia cepacia, Penicillium rugulosum. Burkholderia sp., Bacillus sp	Gluconic acid	Kumari *et al*., 2008, Puente M.E, 2009
Rhizobium leguminosarum, Rhizobium meliloti, Bacillus firmus, Enterobacter intermedium, Pseudomonas cepacia, Burkholderia sp., Acinetobacter sp.	2-Ketogluconic acid	Hoon *et al*., 2003, Leandro M *et al*.,2015
Bacillus liqueniformis, Bacillus amyloliquefaciens	Lactic acid	P. Vazquez *et al*., 2000
Bacillus liqueniformis, Bacillus amyloliquefaciens	Isovalericacid	P. Vazquez *et al*., 2000
Bacillus liqueniformis, Bacillus amyloliquefaciens	Isobutyric acid	P. Vazquez *et al*., 2000
Bacillus liqueniformis, Bacillus amyloliquefaciens	Acetic acid	P. Vazquez *et al*., 2000
Pseudomonas sp., Serratiamarcescens, A. niger, P. simplicissimum	Citric acid	Chen *et al*.,2006
Bacillus megaterium	Propionic acid	Chen *et al*., 2006
Aspergillus flavus, A. niger, Penicillium canescens	Oxalic, citric, gluconic succinic	Maliha *et al*., 2004
A. niger, Burkholderia sp.	Succinic	Vazquez *et al*., 2000
Psedomonas nitroreducens	Indole acetic acid	Pemila E *et al*., 2014

Table 3. Phytases producing microorganism

Sources of phytase enzyme	References
Discosia sp.	Rahi *et al*., 2009
Rhizobacteria	Hariprasad and Niranjana, 2009
Rhizobacteria Pigeon	Patel *et al*., 2010
Serratiamarcescens	Hameeda *et al*., 2006
Pseudomonas sp.	Hameeda *et al*., 2006
Pseudomonas putida	Prashant Singh *et al*., 2014
Pseudomonas mendocina	Jose L. Adrio *et al*., 2014
Pseudomonas fluorescens	Jose L. Adrio *et al*., 2014
Bacillus circulans	Hameeda *et al*., 2006
Emericella rugulosa	Yadav and Tarafdar (2007a, 2007b)
Chaetomium globosum	Tarafdar and Gharu, 2006
A.rugulosus	Hayesetal, 2000
Bacillus mucilaginosus	Li *et al*., 2007

Table 4. Acid phosphatase producing microorganisms

Enzyme	Microorganisms	References
Acid phosphatase	*Emericella rugulosa*	Yadav and Tarafdar (2007a, 2007b)
	Serratiamarcenscens	Hameeda *et al*., 2006
	Chaetomiumglobosum	Hameeda *et al*., 2006
	Serratiamarcenscens	Ryu *et al*., 2005
	P. fluorescens	Ryu *et al*., 2005
	Burkholderia cepacia	Unno *et al*., 2005
	Pseudomonas sp.	Richardson *et al*., 2001a, 2001b
	E. cloacae	Bijender Singh *et al*., 2011
	Proteus mirabali	Bijender Singh *et al*., 2011

In in-vitro conditions, P-solubilizing fungi do not lose the P dissolving activity during repeated sub culturing as occurs with the P-solubilizing bacteria. Some fungi in soils are able to traverse long distances more easily than bacteria and hence, may be more important and essential to P solubilization in soils. Generally, the capacity P-Solubilization of fungi stain is more than bacterial stain.

Among filamentous fungi, *Aspergillus and Penicillium* are the most representative genera of P solubilizers. Although strains of Trichoderm**a** and Rhizoctonia solani have also been reported as P solubilizers (Yasser *et al*., 2014; Fenice *et al*., 2000; Khan *et al*., 2002). Some nemato fungi especially Arthrobotrys oligospora *also* have the ability to solubilize phosphate *in **vivo*** as well as *in vitro* **(**Duponnois *et al*., 2006).

Table 5. Cloning of genes involved in mineral phosphate solubilization (MPS)

Microorganism	Gene involve	Features	Reference
Pseudomonas fluorescens F113 and Pf153	Gcd comp, Gad, pqqB	Gluconic acid production	Miller *et al*., 2010
Pseudomonas cepacia	gabY	Produces gluconic acid and solubilizes mineral P in E. coli JM109	Ke Zhaoa *et al*., 2014
Enterobacte agglomerans	pKKY	Solubilizes P in E. coli JM109 Kim *et al.* (1997)	H. Rodrı´guez *et al*., 2006
Rahnella aquatilis	pK1M10	Solubilizes P and produces gluconic acid in E. coli DH5*x*	H. Rodrı´guez *et al*., 2006
Serratia marcescens	pKG3791	Produces gluconic acid and solubilizes mineral P	Krishnaraj and Goldstein 2001
Synechococcus PCC 7942	pcc gene	Synthesizes phosphoenol pyruvate carboxylase	Sagar Chhabra *et al*., 2013
Erwinia herbicola	mps	Produces gluconic acid and solubilizes mineral P in E. coli HB101	Sagar Chhabra *et al*., 2013

Table 6. Growth-promoting substances produced by plant growth-promoting rhizobacteria

Organisms	Growth regulators	References
Pseudomonas putida	ACC deaminase, IAA, siderophore, ammonia, HCN, P-solubilization	Ahemad *et al*., 2012a, Ahemad *et al*., 2012b Ahemad *et al*., 2011
Pseudomonas sp., Pseudomonas fluorescens, Burkholderia glumae	ACC deaminase, IAA, siderophore, ammonia, HCN, P-solubilization	Shaharoona *et al*., 2008 Jiang *et al*., 2008
Bacillus	ACC deaminase, IAA, siderophore, P-solubilization, HCN	Ahmad *et al*., 2008
Azotobacter	IAA, siderophore, P-solubilization, HCN, ammonia production	Ahmad *et al*., 2008 Joseph *et al*., 2007
Klebsiella oxytoca	IAA, siderophore, P-solubilization, HCN, nitrogenase	Ahemad *et al*., 2011
Azotobacter, Fluorescent Pseudomonas, and Bacillus	IAA, siderophore, ammonia, HCN, P-solubilization	Shaharoona *et al*., 2008 Jiang *et al*., 2008 Ahmad *et al*., 2008
Pseudomonas aeruginosa	P-solubilization, IAA, siderophore, HCN, ammonia	Ahemad *et al*., 2012b
Enterobacter asburiae	Siderophore, IAA, P-solubilization	Ahemad *et al*., 2010
Acinetobacter spp	IAA and P-solubilization	Rokhbakhsh-Zamin *et al*., 2011
Rhizobium sp.	IAA, siderophores, HCN, ammonia	Wani *et al*., 2007
Klebsiella sp	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization	Joseph *et al*., 2007
Bacillus, Pseudomons, Azotobacter, Azospirillum, Rhizobium	P-solubilization and IAA	Tank et. al 2003
Stenotrophomonas maltophilia	Nitrogenase activity, phosphate solubilization, IAA, ACC deaminase	Mehnaz et. al 2010

Among the yeasts, only a few studies have been conducted to assess their ability to solubilize phosphate these include Yarrowia lipolytica, Schizosaccharomyces pombe and Pichiafermentans (Vassilev *et al*., 2001). In recent years, the P-solubilizing ability of *actinomycetes* has attracted interest, because this group of soil organisms is not only capable of surviving in extreme environments (e.g. drought, temperature) but also possess other various potential benefits (e.g. production of antibiotics, siderophore and phytohormones compounds) that could simultaneously benefit plant growth (Hamdali and Bouizgarn, 2008; Hamdali and Hafidi, 2008). A study by Hamdali *et al*. (2008) has also suggested that approximately 20% of actinomycetes can solubilize P, including those in the common genera Streptomyces and Micromonospora.

Biodiversity of Actinomycetes

Some *Actinomycetes* also showed P-solubilizing ability and has attracted interest in recent years because this group of soil organisms is not only capable of surviving in extreme environments (e.g. drought, fire, etc) but they also possess other potential benefits (e.g. production of antibiotics and phytohormone-like compounds) that could simultaneously benefit plant growth (Hamdali H and Bouizgarne, 2008). A study by Hamdali *et al*. (2008) has indicated that approximately 20% of actinomycetes can solubilize P, including those in the common genera (*Streptomyces* and *Micromonospora).* A partial list of PSM including various groups is given in Table 1.

Mechanisms of P Solubilization

P is treated by PSM in two different mechanisms namely P solublization, and P mineralization. The detail mechanism of P solubilization (by inorganic P solubilization) and mineralization (by organic P mineralization) are given below.

Inorganic P solubilization

P solubilizing activity is determined by the ability of microbes to release metabolites such as organic acid and inorganic acids. Of the various strategies adopted by microbes, the involvement of low molecular mass organic acids (OA) secreted by microorganisms has been a well-recognized and widely accepted theory as a principal means of P-solubilization, and various studies have identified and quantified organic acids and defined their role in the solubilization process (Marra *et al*., 2012; Khan *et al*., 2010; Maliha *et al*., 2004). Ketogluconic acid, citric acid, fumaric acid, succinic acid, glycolic acid, oxalic acid, malonic acid, malic acid, vanilic acid, etc are some of the organic acids produced by various PSM. Table 2 summarizes the organic acids synthesized by different PSM. The role of organic acids produced by PSM in solubilizing of insoluble phosphate may be due to: (i) lowering the pH of rhizoshere, (ii) by enhancing chelation of the cations (Al^{3+}, Ca^{2+}, Fe^{3+}, Mg^{2+}) bound to P (iii) by competing with P for adsorption sites on the soil; or (iv) by forming soluble complexes with metal ions associated with insoluble P (Al^{3+}, Ca^{2+}, Fe^{3+}). The lowering of pH of the medium by in-vitro conditions, suggest the release of organic acids by the

P-solubilizing microorganisms via the direct oxidation pathway that occurs on the outer face of the cytoplasmic membrane (Whitelaw *et al*., 2000; Khan *et al*., 2014; Zaidi *et al*., 2009). A direct correlation between drop in pH and increase in available P of the culture media has been observed in certain cases. In few others, the degree of solubilization was not always proportional to the decline in pH. The organic acid produced by the microbial metabolism, (mostly by fermentation of organic carbon sources, e.g., glucose), by oxidative respiration or by such organic acids can either directly dissolve the mineral P as a result of anion exchange of phosphate by acid anion or can chelate Fe, Al and Ca ions associated with P.

Different organisms produce different type of organic acids to solubilize P, which chelate the cations (mainly Al and Fe) bound to phosphate, through their carboxylic groups, converting them into soluble forms. Solubilization processes by chelation is one of the most important mechanism. Many aerobic PSM excrete 2-ketogluconic acid and gluconic acid which are powerful chelators of calcium and such PSM are versatile in solubilization of various form of hydroxyapatites, fluorapatites and aluminium phosphates. Humic and fulvic acids released during microbial degradation of plant debris are also good chelators of calcium, iron and aluminium which are present in insoluble phosphates (Stevenson, 2005). *Rhizobium leguminosarum*, have also been reported to solubilize the insoluble P by secreting 2-ketogluconic acid. Bacteria of *Bacillus amyloliquefaciens* can also dissolve phosphate compounds by producing isobutyric acid. Various studies also suggest that strains of *Bacillus* were found to produce mixtures of lactic, isovaleric, isobutyric and acetic acids. However, acidification cannot be presumed as the sole mechanism of inorganic P solubilization because the extent of soluble P released and pH drop donot always correlate. In another report, an HPLC analysis of the culture solution of Pseudomonas, in contrast to the expectation did not detect any organic acid even though the bacterium solubilized insoluble P easily.

Apart from the organic acid (OA) theory, some of the inorganic acids (*Reyes et al*., 2001; Richardson *et al*., 2001) such as HCl, nitric acid (HNO_3) and sulphuric acid (H_2SO_4) are also able to solubilized insoluble soil P. These inorganic acids produced by chemoautotrophs and the H^+ pump, for example, in *Penicillium rugulosum*, have also been reported to solubilize the insoluble P. Bacteria of the genera *Nitrosomonas* and *Thiobacillus* species can also dissolve phosphate compounds by producing nitric and sulphuric acids. Under anaerobic conditions, some PSMs release Hydrogen sulfide (H_2S) which reacts with insoluble ferric phosphate to yield solubilized ferrous sulphate. Additionally, some PSM has capacity to synthesized siderophores and exopolysaccharides (EPSs) and bring out fixed P into soluble form. In 2008, *Yi et al*. studied four bacterial strains of *Enterobacter sp*. (EnHy-401), *Arthrobacter sp*. (ArHy-505), *Azotobacter sp*. (AzHy-510) and *Enterobacter sp*. (EnHy-402), possessing the ability to solubilize P. These PSB produced a significant amount of EPS and demonstrated a strong ability for P-solubilization. However further studies are necessary to understand the relationship between EPS production and phosphate solubilization (Yi *et al*. 2008).

Organic P Mineralization

Another attractive application of P-dissolving enzymes is the mineralization of soil organic P through phytate degradation mediated by the enzyme phytase, which specifically causes release of P from phytic acid. Phytate is a major component of organic P in soil. In 1997, Richardson has also reported solubilization of the insoluble P by phytase enzyme in *Pseudomonas* sp. The soil microorganisam such as *Discosia* sp., *Rhizobacteria* and *Rhizobacteria* have also been reported to solubilize the insoluble P by secreting phytase enzyme (Table 3). Since then several bacterial and a few fungal species have been isolated which can solubilize the insoluble P by secreting phytase enzyme in liquid cultures and soil systems. On the other hand, a number of phosphatase enzymes are released by PSM, phosphomonoesterases are the most abundant and best studied (Nannipieri *et al*., 2011). Table 4 summarizes the acid phosphatase producing microorganisms. Depending on their pH, these enzymes are divided into acid and alkaline phosphomonoesterases and both can be produced by PSM depending upon the external conditions and soil type type (Orquera *et al*., 2008). Typically, acid phosphatases predominate in acid soils, whereas alkaline phosphatases are more abundant in neutral and alkaline soils (Renella *et al*., 2006). In addition, plant roots are also capable of producing acid phosphatases. Regrettably, it is difficult to differentiate between root and PSM produced phosphatases (Richardson *et al*., 2009). Moreover, phosphonatases and C–P lyases are another mechanism of P solubilization by cleaving C-P bond. Phosphonates are ubiquitous organo-P compounds that contain a characteristic C–P bond which is chemically inert and hydrolytically stable. Bacteria have evolved pathways to metabolize these phosphonate compounds (Rodriguez *et al*., 2006). Different mechanisms involved in the solubilization and mineralization of insoluble P by naturally-occurring microbial communities of soils are briefly illustrated in Figure 1.

Genetics of phosphate solubilizing bacteria

Inorganic P solubilization

In most bacteria, mineral phosphate solubilization capacity has been shown to be related to the production of organic acid. The production of organic acids is considered to be the principal mechanism for mineral phosphate solubilization. Goldstein (1996) proposed direct glucose oxidation to gluconic acid (GA) as a major mechanism for mineral phosphate solubilization. Biosynthesis of gluconic acid is carried out by the glucose dehydrogenase (GDH) enzyme and the co-factor, pyrrolo-quinoline quinone (PQQ). In this regard, *E. coli* is used as a vector because it does not produce gluconic acid, because it is not capable of synthesizing apo-glucose dehydrogenase enzyme (GDH) and the cofactor pyrroloquinoline quinone (pqq). The gene MPS from *Erwinia herbicola* (Gram negative bacteria) has been cloned in *E. coli* HB101 that is involved in mineral phosphate solubilization and proposed the direct glucose oxidation to gluconic acid (GA) as a major mechanism for mineral phosphate solubilization. Sequence analysis of this gene suggested its probable involvement in the synthesis of the enzyme pyrroloquinoline quinone (PQQ) synthase, which directs the synthesis of PQQ, a co-factor necessary for the formation of the holoenzyme glucose dehydrogenase (GDH)-PQQ. This enzyme catalyzes the formation of gluconic acid from glucose by the direct oxidation pathway. Following a similar strategy, another type of mineral phosphate solubilizing gene from *Pseudomonas cepacia* was isolated. This gene (*gabY*) involved in mineral phosphate solubilisation, was cloned in *E.coli* JM109 that induced the production of gluconic.

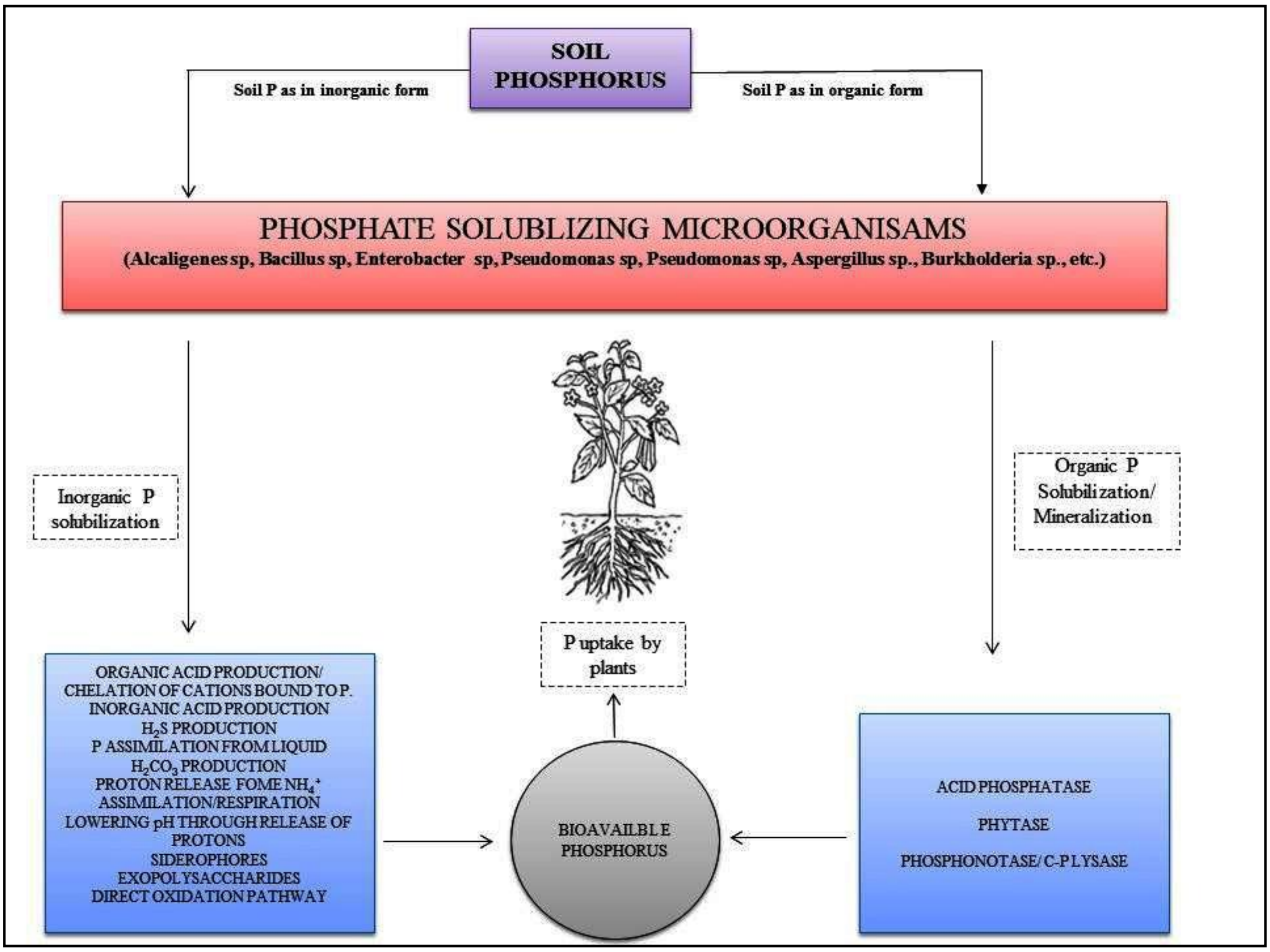

Fig. 1. Schematic representation of mechanism of soil P

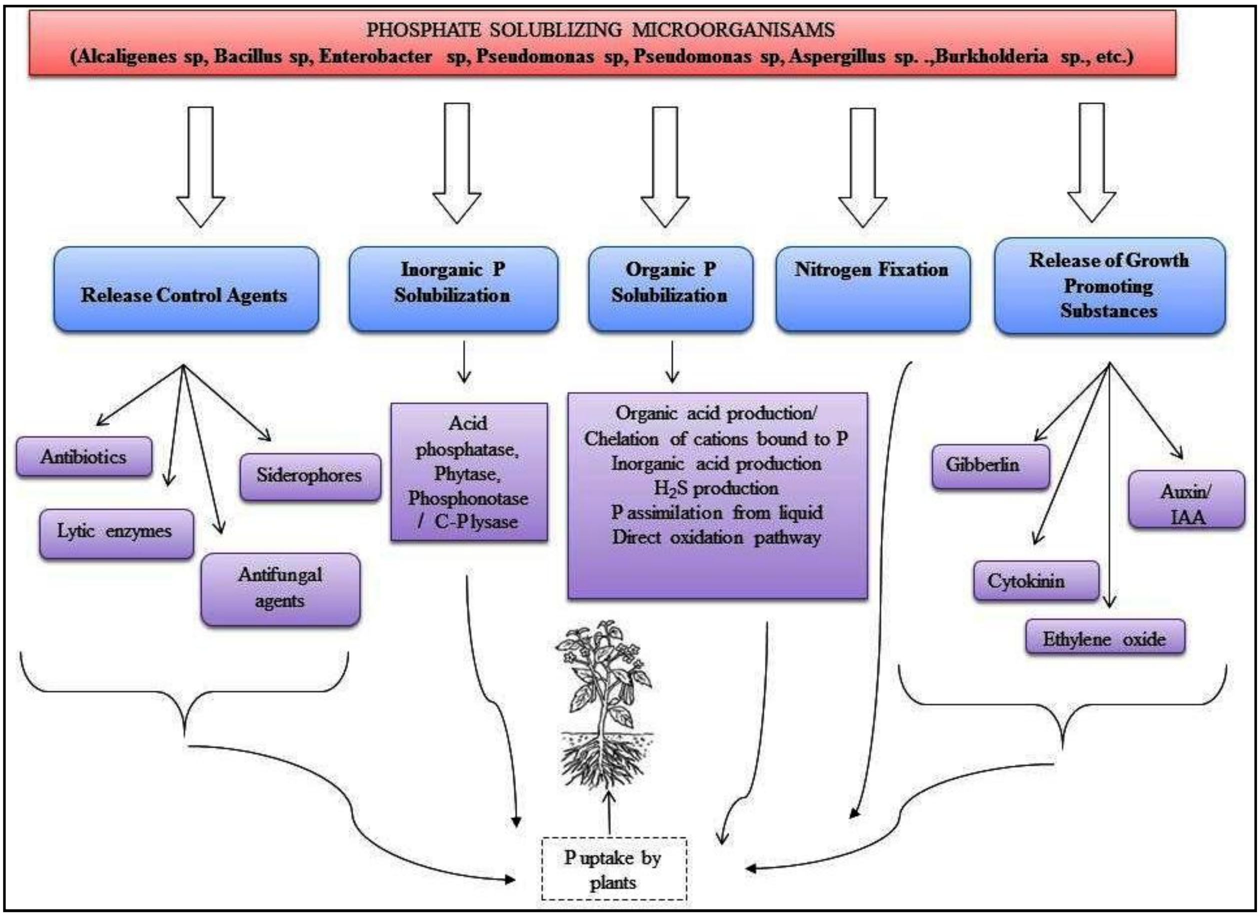

Fig. 2. Possible Mechanisms involved in plant growth

Similarly, the gene *napA* from the soil bacterium Morganella morganii was transferred to Burkholderia cepacia IS-16, a strain used as a biofertilizer as well as P solubilization, by using of vector pRK293 (*Fraga et al*., 2001). Including these, some other genes like *pKKY* and *pKG3791* from *Enterobacte agglomerans* and *Serratia marcescens* respectively are involved in mineral phosphate solublizing. Phosphate solubilizing ability can enhance by genetic manipulation of the genes of phosphate solubilizing bacteria. In addition, gene of *pcc* from *Synechococcus* PCC 7942 appears to be involved in mineral phosphate solublizing when introduced into the rizobacterium pseudomonads fluorescent (Aditi et al., 2009). Table 5 summarizes the genes involved in mineral phosphate solubilization.

Organic P mineralization

Phytate is the major component of organics forms of P in soil. The ability of plants to obtain P directly from phytate is very limited. However, the growth and P nutrition of plants supplied with phytate was improved significantly when they were genetically transformed with the phytase gene (*phyA*) from *Aspergillus niger* (Richardsonet *et al*., 2001a). In addition, several other phosphatase genes have been isolated from *Escherichia coli.* These include: *ushA*, which encodes a 5'-nucleotidase; *agp*, which encodes an acid glucose-1-phosphatase and *cpdB*, encoding the 2'–3' cyclic phosphodiesterase. A gene from *Providencia stuartii* and *Providencia rettgeri* that encodes the 43-kDa acid-hexose phosphatase, as well as a gene cluster involved in the synthesis of specific P-releasing enzyme from organophosphonate substrates (C-P lyase) have also been cloned. These genes may be an interesting source for the further genetic manipulation of soil phosphate solubilizing bacteria. However, study of phytase and phosphatase genes from PSB and their transformation to plant species yet remains to be investigated. Therefore, we need to identify and characterize more PSM with high efficiency for their ultimate application under various field conditions.

Plant growth promoting attributes of PSM

Besides making soluble P accessible for uptake by plants, PSM/or plant growth promoting rhizobacteria (PGPR, having phosphate solubilizing capacity) play an important role in enhancing plant growth through a wide variety of mechanisms. The mode of action of PSM that promotes plant growth includes nitrogen fixation, phytohormones, antibiotics, lytic enzymes and siderophores production (Table.6). However, the modes of action of different PSMs vary depending on the type of host plants and soil type. The mechanisms involved in plant growth promotion by PSM are outlined in Figure 2.

Association of PSB with other microorganisms

Co-inoculation of PSB with *Rhizobium* species stimulated plant growth more profoundly than their inoculation alone. Studies revealed that the cumulative interaction of N_2- fixing (*Rhizobium*) and P-solubilizing (*Bacillus* species) exerted significant increase in yield of legumes. *Rhizobium* in combination with PSB increases phosphorous nutrition by the mobilization of the organic and inorganic phosphate. Zaidi (2006) determined the performance of *Bradyrhizobium* and PSB (*Bacillus subtilis*) on the growth, chlorophyll contents, seed yield, nodulation, grain protein and N and P uptake of green gram plants in P deficient soil. The dual inoculation significantly improved all the parameters against control or single inoculation (Zaidi *et al*., 2006).

In 2007, *Wani et al.* suggested that the increase of 25% compared to control was observed in chickpea seed yield with co-inoculation of *Mesorhizobium ciceri* and *Bacillus* sp. (Khan *et al*., 2007) Various studies revealed that the cumulative interaction of N_2- fixing i.e. *Rhizobium* and P-solubilizing i.e. *Bacillus* species exerted significant increase in yield of legumes by providing balanced plant nutrition (Toro *et al*., 1998) suggested that phosphate solubilizers in addition to enhancing P-concentration in plant tissues were also responsible to improve nodulation and N_2-Fixation. In chickpea, an increase in nodulation, growth, and nutrient content and yield parameters was observed with combined inoculation of *Rhizobium* and PSB (*Pseudomonas striata* and *Bacillus polymyxa*) under greenhouse conditions. In another study, the combined effect of *Bradyrhizobium japonicum* and a PSB (*Pseudomonas sp*.) enhanced the number of nodules, dry weight of nodules, plant yield, soil nutrient availability and uptake of the soybean crop. Integrated effect of PSB and VAM also exhibited a high efficiency to improve the plant growth and nutrition of alfalfa crop. Combined inoculation with *Bacillus circulans* and *Cladosporium herbarum* and VAM fungus resulted in the improved wheat (*Triticum aestivum*) crop yields in nutrient deficient soils. Single inoculation i.e. phosphorous alone, had no significant influence on yield of the crops, but the combined inoculation had shown the significant influence on growth, yield and P uptake by wheat and chickpea (Mukherjee *et al*., 2000). VAM fungi (*Glomus fasciculatum*) and PSB (*Bacillus megaterium var. phosphaticum*) had shown the improved nodulation, nutrient uptake and phosphorous balance, mineral uptake, seed yield and available phosphorous in soil by Soybean plant under field conditions (Dadhich *et al*., 2006). The combination of *Mesorhizobium ciceri* and *Pseudomonas* sp. significantly increased nodule number, dry weight of nodul and root and shoot weights over the control under a glasshouse experiment (Sudeshna Bhattacharjya and Ramesh Chandra, 2013).

PSM as Biofertilizer

The unbalanced use of chemical fertilizers is responsible for reduction in soil fertility and environmental degradation. Biofertilizers can be used to overcome this problem**.** Not all plant-growth promoting bacteria are considered a bofertilizer; if they control plant growth by control of deleterious organisms, they are instead regarded as biopesticides. Microbes have various abilities which could be exploited for better farming practices. Some of them help in combating diseases, while some have the ability to degrade soil complex compounds into simpler forms which are utilized by plants for their growth. They are extremely beneficial in enriching the soil by producing organic nutrients for the soil. To convert insoluble phosphates to a form accessible to the plants, like orthophosphate, is an important trait for a PGPB for increasing plant yields. Some of the other advantages associated with biofertilizers include:

- They are environment friendly, unlike chemical fertilizers that damage the environment.
- They are comparatively low on cost inputs and are light on the pockets of the farmers.

- Their use leads to soil enrichment and the quality of the soil improves with time.
- They do not show immediate results, but the results shown over time are extremely spectacular.
- Biofertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing P, antibiotics, lytic enzymes and siderophores production and stimulating plant growth through the synthesis of growth promoting substances like phytohormones.
- In addition, they also act as antagonists and suppress the incidence of soil borne plant pathogens and thus, help in the biocontrol of diseases.

More recently, phosphate-solubilizing bacteria, such as *Pantoea agglomerans* strain P5, *Microbacterium laevaniformans* strain (P7) and *Pseudomonas putida* strain P13 (Mohammad *et al*., 2009), have been identified to solubilize the insoluble phosphate from organic and inorganic phosphate sources and can be used as a biofertilizer (Pandey *et al*., 2006) NII-0909 Strain of *Micrococcus* sp. has polyvalent properties including phosphate solubilization and siderophore production. *Burkholderia vietnamiensis*, stress tolerant bacteria, produces gluconic and 2-ketogluconic acids, which is involved in phosphate solubilization.

Future prospectus

At a global scale, adverse environmental effects of chemical based P fertilsers and their increasing prices have compelled us to find a sustainable approach for efficient P availability in agriculture. Soil microorganisms are involved in a range of processes that affect P transformation and thus influence the subsequent availability of P (as phosphate) to plant roots. PSM not only provide phosphorous to the plant but at the same time also provide growth-promoting substances (like hormones, vitamins, and amino acids) and other control agents (like antifungal substance, antibiotics, siderophores and lytic enzymes). However, their performance under field conditions is not reliable and therefore needs to be improved. Because microorganisms establish associations with plants and promote plant growth by means of several beneficial characteristics, therefore there is an urgent need for research describing clear definition of bacterial traits which are useful and necessary in different environmental conditions for plants, so that optimal bacterial strains can either be selected and/or improved. Greater attention should be paid to studies and application of new combinations of phosphate solubilizing bacteria and other PGPR for improved results. With the help of genetic engineering, introduction of genes involved in soil phosphate solubilization (both organic and inorganic) in natural rhizosphere bacteria is a very attractive and modern approach for improving the capacity of microorganisms to work as inoculants. 16S rRNA gene sequences along with next-generation sequencing provide nailing application for assessment of diversity surrounding particular traits or functional groups of microorganisms. Genetic manipulation by recombinant DNA technology, proteomic and transcriptomic characterization of microbial genotypes seem to offer a feasible approach for obtaining improved strains. Cloning of genes involved in mineral phosphate solubilization, such as those influencing the synthesis of organic acids, as well as phosphatase encoding genes, would be the first step in such a genetic manipulation program. Sub-cloning of these genes in appropriate vectors and their transfer and expression (or over-expression) in target host strains could be a successful procedure for improving the phosphate solubilization capabilities of selected strains. Recipient strains should be selected either for the expression of a certain phosphate solubilizing activity, which is to be improved, or for the presence of some other important trait involved in plant growth promotion that would favorably complement the potential to release P from insoluble substrates. Additionally, the use of powerful and species-specific promoters, which could be activated under the specific environmental conditions of soil, is another interesting approach to successful gene expression in the engineered strain. In brief, biotechnology and molecular biology together can search for region specific microbial strains which can be used as a potential plant growth promoter to achieve desired production of strains for biofertilizer. On the other hand, the putative risk involved in the release of genetically engineered microorganisms in soil is a matter of controversy, in particular with regard to the possibility of horizontal transfer of the inserted DNA to other soil microorganisms.

Therefore, the use of genetic reporter gene, such as bioluminescence genes, or green fluorescent protein genes is crucial in studying the fate and survival of the strain in soil. Furthermore, scientists need to address certain issues like, what should be an ideal and universal delivery system, how to improve the efficacy of biofertilizers, how to stabilize these microbes in soil systems, how to control production cost and how nutritional and root exudation aspects could be controlled in order to get maximum benefits from PSM application. To conclude, vast and focused studies should be done to examine the natural biodiversity of soil organisms and their optimization. Biotechnologist and soil microbiologists have a great responsibility to find new ways to improve soil fertility without applying the chemical P fertilizers under different agro-climatic regions of the world and deliver an eco-friendly environment. However, before using of microbial isolates as a biofertilizer, it should be investigated for crop productivity and its quality.

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