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A Review

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Sustainable way for enhancing phosphorus efficiency in agricultural soils through phosphate solubilizing microbes

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Summary

Phosphorus is the second important key element after nitrogen as a nutrient in terms of quantitative plant requirement. Although phosphorus is abundant in soils (organic and inorganic forms), its availability is restricted as it occurs mostly in insoluble forms. The phosphorus content in soil is about 0.05 per cent (w/w) but only 0.1 per cent of the total phosphorus is available to plant because of poor solubility and its fixation in every type of soil. An adequate supply of phosphorus during early phase of plant development is important for laying down the primordia of plant parts. It plays significant role in root ramification, thereby imparting vitality to plant. It also helps in seed formation and in early maturation of crops. Poor availability or deficiency of phosphorus markedly reduces plant size and growth. Phosphorus accounts about 0.2 - 0.8 per cent of the plant dry weight. To satisfy crop requirements, phosphorus is usually added to soil as chemical fertilizer, however, synthesis of chemical fertilizer is highly energy intensive processes, and has long term impacts on the environment in terms of eutrophication, soil fertility depletion, carbon footprint. Moreover, plants use only a small amount of phosphorus, because about 80–90 per cent of added phosphorus is precipitated by metal–cation complexes, and rapidly fixed in soils. Such environmental concerns have led to the search for sustainable way of phosphorus nutrition of crops. In this regards phosphate–solubilizing microorganisms have been seen as best eco–friendly means for phosphorus nutrition of crop. Although, several bacterial (*Pseudomonas* and *Bacilli*) and fungal strains (*Aspergillus* and *Penicillium*) have been identified as PSM. Their performance under *in situ* conditions is not reliable and therefore, needs to be improved by using co-inoculation techniques. This review focuses on the diversity of PSM, mechanism of P solubilization, role of various phosphatase, impact of various factors on solubilization, the present and future scenario of their use and potential for application of this knowledge in managing a sustainable agricultural system.

Key words : Soil phosphorus, PSM, Solubilization, Biodiversity, Biofertilizers, Siderophores, TCP, Organic acids

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Introduction

Phosphorus is the most important element in the nutrition of plants, next to nitrogen. It plays various important role in metabolic processes in plant, including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration (Khan *et al.*, 2010) and nitrogen fixation in legumes (Saber *et al.*, 2005). Although P is abundant in soils in both inorganic and organic forms, but it is a major limiting factor for plant growth, as it is in unavailable form for root uptake. Inorganic phosphorus occurs in soil, mostly in insoluble complexes. These insoluble, precipitated forms cannot be absorbed by plants direclty (Rengel and Marschner, 2005). Organic matter is also an important reservoir of immobilized P that accounts for 20–

80 per cent of P in soils (Richardson, 1994). Only 0.1 per cent of the total P exists in a soluble form is available for plant uptake (Zhou *et al.,* 1992) because of its fixation into an unavailable form. The P fixation is used to describe reactions that remove available phosphate from the soil solution into the soil solid phase (Barber, 1995). There are two types of reactions (a) phosphate sorption on the surface of soil minerals and (b) phosphate precipitation by free Al^{3+} and Fe^{3+} in the soil solution (Havlin *et al.,* 1999). It is for this reason that soil P becomes fixed and available P levels have to be supplemented on agricultural soils by adding chemical P fertilizers, which enhance cost of agricultural production and also impose adverse impacts on overall soil health and degradation of terrestrial (Tilman *et al.,* 2001). The repeated and injudicious applications of chemical P fertilizers, leads to the loss of soil fertility (Gyaneshwar *et al*., 2002) by disturbing microbial diversity and consequently reducing yield of crops. The long-term effect of different sources of phosphatic fertilizers on microbial activities includes inhibition of substrate-induced respiration by streptomycin sulphate (fungal activity) and actidione (bacterial activity) and microbial biomass carbon (Bolan *et al.,* 1996). Similarly, the application of triple superphosphate (94 kg/ ha) has shown a substantial reduction in microbial respiration and metabolic quotient (qCO_2) (Chandini and Dennis, 2002).

The efficiency of applied phosphorus fertilizers rarely exceeds 30 per cent due to its fixation, either in the form of iron/ aluminium phosphate in acidic soils (Norrish and Rosser, 1983) or in the form of calcium phosphate in neutral to alkaline soils (Lindsay *et al.,* 1989). It has been suggested that the accumulated phosphorus in soils would be sufficient to sustain maximum crop yields. A major characteristic of phosphorus biogeochemistry is that only 1 per cent of the total soil phosphorus (400–4,000 kg P/ ha in the top 30 cm) is incorporated into living plant biomass during each growing season (10–30 kg P/ha), reflecting its low availability for plant uptake (Blake *et al.,* 2000). The realization of all these potential problems associated with chemical fertilizers together with the enormous cost involved in their manufacturing, has led to the search for economically feasible alternative for improving crop production in low or P-deficient soils (Zaidi *et al.,* 2009). The use of microbial inoculants possesing P-solubilizing activities in soils is considered as an environmental-friendly alternative to further applications of chemical based P fertilizers.

Phosphate fertilizers facts and current trends :

In India, deposits of sufficiently enriched phosphatic rocks are imports 2 million tons of rock phosphate annually. About 98 per cent of cropland in India is deficient in available forms of soil phosphorus and only 1–9 per cent has high phosphorus. Intensive cropping pattern has also resulted in widespread deficiency of phosphorus. Although various amendments are available for management of phosphorus in different soil, but all are costlier and practically difficult. Thus, even if the total soil phosphorus is high and also if fertilizers are applied regularly, pH dependent chemical fixation, determines the quantity of available phosphorus. The holistic phosphorus management practices involves a series of strategies like manipulation of soil and rhizosphere processes, development of phosphorus efficient crops and improving phosphorus recycling efficiency. Microbially mediated P management is a cost effective approach for sustainable development of agricultural. Microorganisms are an integral component of the soil phosphorus cycle. It is important for the transfer of P between different pools of soil P. Phosphate solubilizing microorganisms (PSM) through various mechanisms of solubilization and mineralization are able to convert inorganic and organic soil P, respectively (Khan *et al*., 2009a) into the bio-available form facilitating uptake by plant roots. Hence, it is imperative to better understand the plant-soil-microbial P cycle with the aim of reducing reliance on chemical P fertilizers.

Microbial intervention of PSM seems to be an effective way to enhance the phosphorus availability in soil. However, P-solubilization in soil is much more difficult to study than solubilization of phosphorus in broth culture. The crops respond differently to the inoculation of PSMs and are dependent on several factors such as the soil temperature, moisture, pH, salinity, and source of insoluble P, method of inoculation, the energy sources and the strain of microorganism used. Hence, study of PSM activity in correlation with these factors has to be done extensively before PSM can be used as a biofertilizer with promising results. The successful implementation of this approach has already been demonstrated in the fields but to a limited extent. Therefore, the large scale use of this technology is required. The organisms involved in phosphorus cycling in soils are highly varied. However, more than 99 per cent of soil microorganisms have not been cultured successfully (Torsvik and Ovreas, 2002). Therefore, culture-independent methods are required to study the function and ecology of microbes involved in P cycling in soils. Looking at the possible avenues with exploring these environmental friendly microorganisms, it is necessary to study the composition and dynamics of these microbial populations to for better understanding of soil PSM diversity and P uptake by plants.

PSM inoculations and their acceptance by farming communities :

The major concern is why P is unavailable to plants in soluble form when there is abundance of potential P-solubilizing microbes in soils? Many reasons have been suggested for such variations in the effectiveness of microbial inoculations and their consequent effect on plant growth: (i) the poor survival and colonization of inoculated P-solubilizing microbes in the rhizosphere, (ii) strong competition from native microbial communities which lead to exclusion of introduced strains, (iii) physico-chemical properties of soils, (iv) signal compounds released by different plant genotypes, (v) availability of inadequate nutrients in the rhizosphere to produce enough organic acids, (vi) variation in the persistence of P-solubilizing activity, and (vii) genetic instability among inoculated strains. Moreover, despite the promising results obtained *in vitro*, the microbial inoculants are not so popular among farmers. Therefore, efforts should be made to make the use of natural resources like P-solubilizing microbes in order to reduce the environmental pollution besides promoting the growth of plants in different agro-climatic regions.

Isolation of PSM :

Solubilization of insoluble P by microorganisms was reported by Pikovskaya (1948). These organisms are ubiquitous but vary in density and phosphate solubilizing ability from soil to soil. In soil, P solubilizing bacteria constitute 1-50 per cent and fungi 0.1-0.5 per cent of the total respective population. During the last two decades knowledge on phosphate solubilizing microorganisms increased significantly (Richardson, 2001). Several strains of bacterial and fungal species have been investigated in detail for their phosphate-solubilizing capabilities. Typically such microorganisms have been isolated using cultural procedures with species of *Pseudomonas* and *Bacillus* bacteria (Illmer and Schinner, 1992) and *Aspergillus* and *Penicillium* fungi being predominant (Wakelin *et al*., 2004). They are isolated from rhizosphere, non-rhizosphere soils, rhizoplane, phyllosphere, rock P deposited area soil and even from stressed soils using serial plate dilution method or by enrichment culture technique (Zaidi *et al*., 2009).

In 1948, when Pikovskaya suggested that microbes could dissolve non-readily available forms of soil P to plants, numerous methods and media, such as Pikovskaya (Pikovskaya, 1948), bromophenol blue dye method (Gupta *et al*., 1994) and National Botanical Research Institute P medium (Nautiyal, 1999) have been proposed. The source of insoluble phosphate in the culture media to isolate PSM is a major issue of controversy regarding the isolation of PSM. Commonly used selection factor for this trait, tricalcium phosphate (TCP), is relatively weak and unreliable factor for isolating and testing PSM for enhancing plant growth. The use of TCP usually yields many isolates of “supposed” PSM. When these isolates are further tested for direct contribution of phosphorus to the plants, only a very few are proved true PSM. Therefore, other compounds should also be tested. Here multiple sources of insoluble phosphate are recommended. The selection of potential PSM will depend on the type of soil, where the PSM will be used. Adding calcium phosphate compounds (including rock phosphates) for alkaline soils, iron/aluminium phosphate compounds for acidic soils, and phytates for soils rich in organic P is suggested by Bashan *et al.* (2013a and b).

Both bacterial and fungal strains exhibiting P solubilizing activity are detected by the formation of clear halo zone around their colonies. Production of a halo on a solid agar medium should not be considered the sole test for P solubilization. Therefore, an additional test in liquid media should be performed and the few isolates that are obtained after such rigorous selection should be further tested for abundant production of organic acids and the isolates complying with these criteria should be tested on a plant as the ultimate test for potential P solubilization (Bashan *et al*., 2013a). The viable microbial preparations possessing P-solubilizing activity are generally termed as microphos (Zaidi *et al*., 2009). The phosphate-solubilizing microbes showing greater solubilization (both qualitatively and quantitatively) of insoluble P under *in vitro* conditions are selected for field trials prior to production in bulk for ultimate transmission as a biofertilizer. Protocol for isolation and effective

Soil sample collection
↓
Serial dilution
↓
Inoculation on media (pour plate/streaking) having different source of insoluble P depending on soil type
↓
Clear Zone around colony growth indicates PSM activity
↓
Additional test in liquid media to assay P dissolution
↓
Test isolates for abundant production of organic acids
↓
Pure culture by re-inoculation
↓
Study of the morphology and colony characterization and biochemical tests
↓
Screening of best inoculants in terms of P solubilizing activity
↓
Identification at genetic level (Molecular characterization)
↓
Test on a model plant
↓
Development of microphos
↓
Green house trials
↓
Field trials
↓
Standardization (quality control)
↓
Commercial biofertilizer

Fig. 1 : Protocol for isolation and development of effective inoculants of PSM

inoculants development of PSM has been shown in Fig. 1.

Biodiversity of P solubilizers :

A huge number of microbial species exhibit P solubilization capacity; these include bacteria, fungi, actinomycetes and even algae. In addition to *Pseudomonas* and *Bacillus*, other bacteria reported as P-solubilizers include *Rhodococcus, Arthrobacter, Serratia, Chryseobacterium, Gordonia, Phyllobacterium, Delftia* sp. (Chen *et al.,* 2006), *Azotobacter* (Kumar *et al*., 2001), *Xanthomonas* (De Freitas *et al*., 1997), *Enterobacter, Pantoea, and Klebsiella* (Chung *et al.,* 2005), *Vibrio proteolyticus, Xanthobacter agilis* (Vazquez *et al.,* 2000). Furthermore, symbiotic nitrogenous *rhizobia*, which fix atmospheric nitrogen into ammonia, have also shown P solubilizing activity (Zaidi *et al*., 2009), For instance, *Rhizobium leguminosarum bv. Trifolii* (Abril *et al.*, 2007), and *Rhizobium* species nodulating *Crotalaria* species (Sridevi *et al.,* 2007) improved plant P-nutrition by mobilizing inorganic and organic P.

In soil, P-solubilizing fungi constitute about 0.1–0.5 per cent of total fungal populations (Kucey, 1983). Moreover, P-solubilizing fungi do not lose the P dissolving activity upon repeated sub culturing under laboratory conditions as occurs with the P-solubilizing bacteria (Kucey, 1983). 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 than bacteria and consequently exhibit greater P-solubilizing activity (Venkateswarlu *et al*., 1984). Among filamentous fungi that solubilize phosphate, the genera *Aspergillus* and *Penicillium* (Khan and Khan, 2002) are the most representative although strains of *Trichoderma* (Altomare *et al*., 1999) and *Rhizoctonia solani* (Jacobs *et al.*, 2002) have also been reported as P solubilizers. A nemato-fungus *Arthrobotrys oligospora also* has the ability to solubilize phosphate *in vivo* as well as *in vitro* (Duponnois *et al.*, 2006). Among the yeasts, only a few studies have been conducted to assess their ability to solubilize phosphate these include *Yarrowia lipolytica* (Vassilev *et al*., 2001), *Schizosaccharomyces pombe* and *Pichia fermentans*. As more studies are conducted, a wider diversity of phosphate-solubilizing filamentous fungi is expected to be described. Of those identified, many are commonly found in agricultural soils such as *Penicillium* sp., *Mucor* sp. and *Aspergillus* sp. which has been shown to increase plant growth by 5–20 per cent after inoculation (Gunes *et al*., 2009).

The P-solubilizing ability of actinomycetes has attracted interest in recent years because this group of soil organisms is not only capable of surviving in extreme environments but also possess other potential benefits (e.g. production of antibiotics and phytohormone) that could simultaneously benefit plant growth (Hamdali *et al*., 2008a and b). A study by Hamdali *et al.* (2008a) has indicated that approximately 20 per cent 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.

In addition to bacteria, fungi and actinomycetes, algae such as cyanobacteria and mycorrhiza have also been reported to show P solubilization activity. The interactive effects of arbuscular mycorrhizal fungi (AMF) and rhizobacteria on the growth and nutrients uptake of *Sorghum bicolor* were studied in acid and low availability phosphate soil. The microbial inocula consisted of the AMFs *Glomus manihotis* and *Entrophospora colombiana*, PSB *Pseudomonas* sp., results indicated that the interaction of AMF and the selected rhizobacteria has a potential to be developed as biofertilizers in acid soil. The potential of dual inoculation with AMF and rhizobacteria needs to be further evaluated under different crop and agroclimatic conditions, particularly in the field

Table 1 : Biodiversity of PSM

Bacteria	*Alcaligenes* sp., *Aerobactor aerogenes, Achromobacter* sp., *Actinomadura oligospora, Agrobacterium* sp., *Azospirillum brasilense, Bacillus* sp., *Bacillus circulans, B.cereus, B. fusiformis, B. pumils, B. megaterium, B. mycoides, B. polymyxa, B. coagulans B. chitinolyticus, B. subtilis, Bradyrhizobium* sp., *Brevibacterium* sp., *Citrobacter* sp., *Pseudomonas* sp., *P putida, P. striata, P. fluorescens, P. calcis, Flavobacterium* sp., *Nitrosomonas* sp., *Erwinia* sp., *Micrococcus* sp., *Escherichia intermedia, Enterobacter asburiae, Serratia phosphoticum, Nitrobacter* sp., *Thiobacillus ferroxidans, T. thioxidans, Rhizobium meliloti, Xanthomonas* sp.
Fungi	*Aspergillus awamori, A. niger, A. tereus, A. flavus, A. nidulans, A. foetidus, A. wentii. Fusarium oxysporum, Alternaria teneius, Achrothcium* sp. *Penicillium digitatum, P lilacinium, P balaji, P. funicolosum, Cephalosporium sp. Cladosprium* sp., *Curvularia lunata, Cunnighamella, Candida* sp., *Chaetomium globosum, Humicola inslens, Humicola lanuginosa, Helminthosporium* sp., *Paecilomyces fusisporous, Pythium* sp., *Phoma* sp., *Populospora mytilina, Myrothecium roridum, Morteirella* sp., *Micromonospora* sp., *Oideodendron* sp., *Rhizoctonia solani, Rhizopus* sp., *Mucor* sp., *Trichoderma viridae, Torula thermophila, Schwanniomyces occidentalis, Sclerotium rolfsii.*
Actinomycetes	*Actinomyces,, Streptomyces*
Cyanobacteria	*Anabena* sp., *Calothrix braunii, Nostoc* sp., *Scytonema* sp.,
VAM	*Glomus fasciculatum.*

(Widada *et al.*, 2007). Hence the studies have shown that the diversity of the PSM's is highly varied in different ecological niches and there is ample scope to identify many new potent isolates in coming times.

Mechanisms of P- solubilization by PSM :

The P- solubilization mechanisms employed by soil microorganisms include: (1) release of complexing or mineral dissolving compounds e.g. organic acid anions, siderophores, protons, hydroxyl ions, CO_2, (2) liberation of extracellular enzymes (biochemical P mineralization) and (3) the release of P during substrate degradation (biological P mineralization) (McGill and Cole, 1981). Therefore, microorganisms play an important role in all three major components of the soil P cycle. Release of P immobilized by PSM primarily occurs when cells die due to changes in environmental conditions, starvation or predation. Environmental changes, such as drying–rewetting or freezing–thawing, can result in so-called flush-events, a sudden increase in available P in the solution due to an unusually high proportion of microbial cell lysis (Butterly *et al.*, 2009). Grierson *et al.* (1998), found that about 30–45 per cent of microbial P (0.8–1 mg kg^{-1}) was released in a sandy spodosol in an initial flush after drying–rewetting cycles within the first 24 hour.

Inorganic P solubilization :

by P-solubilizing microorganisms occurs mainly by organic acid production (Table 2), either by:

- lowering of the pH, or
- by enhancing chelation of the cations bound to P
- by competing with P for adsorption sites on the soil
- by forming soluble complexes with metal ions associated with insoluble P (Ca, Al, Fe) and thus, P is released.

The lowering in pH of the medium suggests the release of organic acids by the P-solubilizing microorganisms (Maliha *et al.*, 2004) via the direct oxidation pathway that occurs on the outer face of the cytoplasmic membrane (Zaidi *et al*., 2009). These acids are the product of the microbial metabolism (Trolove *et al.*, 2003).

The monovalent anion phosphate $H_2PO_4^-$ is a major soluble form of inorganic phosphate, which usually occurs at lower pH. However, as the pH of the soil increases the divalent and trivalent forms of P (HPO_4^{-2} and HPO_4^{-3}, respectively) occur. Thus, the synthesis and discharge of organic acid by the PSM strains into the surrounding environment acidify the cells and their surrounding environment that ultimately lead to the release of P ions from the P mineral by H^+ substitution for the cation bound to phosphate (Goldstein, 1994). The prominent acids released by PSM in the solubilization of insoluble P are gluconic acid (Bar-Yosef *et al*., 1999), oxalic acid, citric acid (Kim *et al*., 1997), lactic acid, tartaric acid, aspartic acid (Venkateswarlu *et al*., 1984). Evidence from an abiotic study using HCl and gluconic acid to solubilize P also indicated that chelation of

Table 2 : Important PSM, their ecological niches and organic acids produced

Organism	Ecological niche	Predominant acids produced	Reference
PSB	Soil and phosphate bearing rocks	Not determined	Pikovskaya, 1948
PSB	Bulk and rhizospheric soil	Not determined	Gerretson, 1948
Escherichia freundii	Soil	Lactic	Sperber, 1958a, b
Aspergillus niger, Penicillium sp.	Soil	Citric, glycolic, succinic, gluconic, oxalic, lactic	Sperber, 1958a, b
Bacillus megaterium, Pseudomonas sp., *Bacillus subtilus*	Rhizospheric soil	Lactic, malic	Taha *et al*., 1969
Arthrobacter sp., *Bascillus* sp., *Bacillus firmus B-7650*	Wheat and cowpea rhizosphere	Lactic, citric	Bajpai and Sundara Rao, 1971
Aspergillus sp., *Penicillium* sp., *Chaetomiumnigricolor*	Lateritic soil	Oxalic, Succinic, Citric, 2-ketogluconic	Banik and Dey, 1983
A. japonicus, A. foetidus	Indian Rock phosphate	Oxalic, citric, gluconic succinic, tartaric acid	Singal *et al*., 1994
P. radicum	Rhizosphere of wheat roots	Gluconic	Whitelaw *et al.*, 1999
Enterobacteragglomerans	Wheat rhizosphere	Oxalic, citric	Kim *et al*., 1997
Penicillium rugulosum	Venezuelan phosphate rocks	Citric, gluconic acid	Reyes *et al*., 2001
Enterobacter intermedium	Grass rhizosphere	2-ketogluconic	Hwangbo *et al.*, 2003
P. fluorescens	Root fragments and rhizosphere of oil palm trees	Citric, malic, tartaric, gluconic	Fankem *et al*., 2006
Aspergillus niger	Tropical and subtropical soil	Gluconic, oxalic	Chuang *et al.*, 2007

Al^{3+} by gluconic acid may have been a factor in the solubilization of colloidal Al phosphate (Whitelaw *et al.*, 1999). Organic acids produced by P-solubilizing microorganisms can be detected by high performance liquid chromatography and enzymatic methods (Whitelaw, 2000). However, acidification does not seem to be the only mechanism of solubilization, as the ability to reduce the pH in some cases did not correlate with the ability to solubilize mineral P (Subba Rao, 1982). Altomare *et al.* (1999) investigated the capability of the plant-growth promoting and biocontrol fungus *T. harzianum* to solubilize *in vitro* insoluble minerals including rock phosphate. Organic acids were not detected in the culture filtrates and hence, the authors concluded that acidification was probably not the major mechanism of solubilization as the pH never fell below 5. The phosphate solubilizing activity was attributed both to chelation and to reduction processes. Although, organic acid has been suggested as the principal mechanism of P solubilization, the solubilization of insoluble P by inorganic acid (e.g. HCl) has also been reported, although HCl was able to solubilize less P from hydroxyl-apatite than citric acid or oxalic acid at same pH (Kim *et al.*, 1997). Bacteria of the genera *Nitrosomonas* and *Thiobacillus* species can also dissolve phosphate compounds by producing nitric and sulphuric acids (Azam and Memon, 1996).

According to the sink theory, P-solubilizing organisms remove and assimilate P from the liquid and hence, activate the indirect dissolution of calcium phosphate by removal of P from liquid culture medium. The other mechanism is the production of H_2S, which react with ferric phosphate to yield ferrous sulphate with release of phosphate (Swaby and Sperber, 1958).

H^+ excretion originating from NH_4^+ assimilation as proposed by Parks *et al.* (1990) could be the alternative mechanisms of P solubilization. An HPLC analysis of the culture solution of *Pseudomonas* sp., in contrast to the expectation, did not detect any organic acid while solubilization occurred (Illmer and Schinner, 1995). They also reported that the most probable reason for solubilization without acid production is the release of protons accompanying respiration or NH_4^+ assimilation. Krishnaraj *et al.* (1998) have proposed a model highlighting the importance of protons that are pumped out of the cell to be the major factor responsible for P solubilization. Here direct role of organic or inorganic acids has been ruled out. For some microorganisms, NH_4^+ driven proton release seems to be the sole mechanism to promote P solubilization. Asea *et al.* (1988) tested two fungi, *Penicillium bilaii* and *Penicillium fuscum*, for their ability to solubilize phosphate rock in the presence of NH_4^+ or without N addition, and showed that only *P. bilaii* maintained the ability to decrease the pH and mobilize P when no N was supplied. In a study of *Pseudomonas fluorescens*, the form of C supply rather than N supply had the greatest effect on proton release (Park *et al.*, 2009). Further, the involvement of the H^+ pump mechanism in the solubilization of small amounts of P in *Penicillium rugulosum* is reported (Reyes *et al.*, 1999). This indicates that for different species, different mechanisms are responsible for proton release, only partly depending on the presence of NH_4^+.

Goldstein (1995) suggested that extracellular oxidation via direct oxidation pathway may play an essential role in soils where calcium phosphates provide a significant pool of unavailable mineral phosphorus. This has been confirmed by some researchers (Song *et al.*, 2008) by biochemical analysis of lowering of pH in insoluble P solubilization by *Burkholderia cepacia*.

Organic P solubilization is also called mineralization of organic phosphorus. Mineralization of soil organic P (Po) plays an imperative role in phosphorus cycling of a farming system. Organic P may constitute 4–90 per cent of the total soil P (Khan *et al.*, 2009b). Such P can be released from organic compounds in soil by enzymes.

Non-specific acid phosphatases (NSAPs), which dephosphorylate phospho-ester or phosphoanhydride bonds of organic matter. Among the variety of phosphatase enzyme classes released by PSM, phosphomonoesterases (also called phosphatases) are the most abundant and best studied (Nannipieri *et al.*, 2011). Depending on their pH optima, these enzymes are divided into acid and alkaline phosphomonoesterase and both can be produced by PSM depending upon the external conditions (Kim *et al.*, 1998; Jorquera *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). Although plant roots can produce acid phosphatases they rarely produce large quantities of alkaline phosphatases, suggesting that this is a potential niche for PSM (Criquet *et al.*, 2004). It is difficult to differentiate between root- and PSM-produced phosphatases (Richardson *et al.*, 2009a and b) but some evidence suggests that phosphatases of microbial origin possess a greater affinity for Po compounds than those derived from plant roots (Tarafdar *et al.*, 2001). The relationship between PSM introduced into soil, phosphatase activity and the subsequent mineralization of Po still remains poorly understood (Chen *et al.*, 2003) .

Phytases, which specifically cause release of P from phytate. In its basic form, phytate is the primary source of inositol and the major stored form of P in plant seeds, and is a major component of organic P in soil. Although the ability of plants to obtain P directly from phytate is very limited, yet the growth and P-nutrition of Arabidopsis plants supplied with phytate was significantly improved when they were genetically transformed with the phytase gene (phy A) derived from *Aspergillus niger* (Richardson *et al.*, 2001). This led to an increase in P-nutrition to such an extent that the growth and P-content of the plant was equivalent to control plants supplied with inorganic P. Hence, microorganisms are key driver in regulating the mineralization of phytate in soil and their presence within the rhizosphere may compensate for a plants inability to otherwise acquire P directly from phytate (Richardson and Simpson, 2011).

Phosphonatases and C–P lyases, that cleave the C–P bond of organophosphonates (Rodriguez *et al*., 2006). It occurs through different mechanisms and there is considerable variation amongst the organisms. Each organism can act in one or more than one way to bring about the solubilization of insoluble P. Different mechanisms involved in the solubilization and mineralization of insoluble P by naturally-occurring microbial communities of soils is briefly illustrated in Fig. 2.

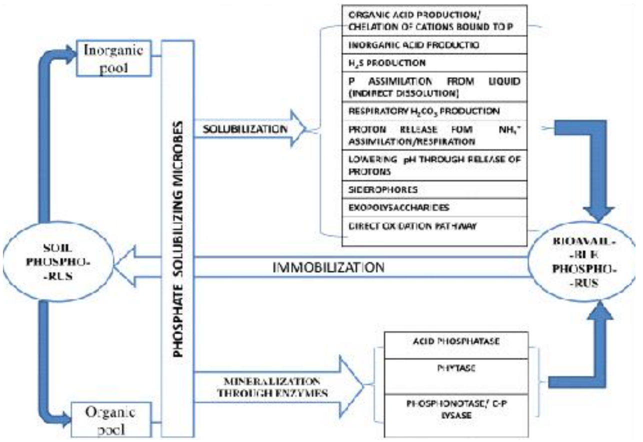

Fig. 2 : **Schematic representation of mechanism of soil P solubilization/mineralization and immobilization by PSM**

Role of siderophores in P- solubilization :

Siderophores are complexing agents that have affinity for iron and are produced by almost all microorganisms in response to iron deficiency. Thus, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. There are nearly 500 known siderophores, which being exclusively used by the microbial species and strains that produce them. Studies have reported the release of siderophores from PSM (Hamdali *et al*., 2008a); however, siderophore has not been widely implicated as a P-solubilization mechanism. Considering the dominance of mineral dissolution over ligand exchange by organic acid anions as a P-solubilizing mechanism (Parker *et al*., 2005), the potential role of siderophores in enhancing P availability should be obvious.

Role of EPS in P-solubilization :

Recently the role of polysaccharides in the microbial mediated solubilization of P was assessed. Microbial exopolysaccharides (EPSs) are polymers consisting mainly of carbohydrates excreted by some bacteria and fungi onto the outside of their cell walls. Their composition and structures are very varied; they may be homo- or heteropolysaccharides and may also contain a number of different organic and inorganic substituents (Sutherland, 2001). 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 TCP (tri calcium phosphate), were used to assess the role of exopolysaccharide (EPS) in the solubilization of P by Yi *et al*. (2008). These phosphate solubilizing bacteria 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.

Future prospects :

Despite the different ecological niches and multiple functional properties, P-solubilizing microorganisms have yet to fulfill their promise as commercial bio-inoculants. Current developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems. Although significant studies related to PSM and their role in agriculture have been done over the last few decades, the required technique remains in its infancy. Nevertheless with an awareness of the limitations of existing methods, a reassessment can be expected, so that the use of PSM as potential biofertilizers in different soil conditions becomes a reality.

Enhancement in the use of PSM is one of the newly emerging options for meeting agricultural challenges imposed by the still-growing demand for food. Thus, obtaining high yields is the main challenge for agriculture. In addition to this, in recent years both producers and consumers have increasingly focused on the health and quality of foods, as well as on their organoleptic and nutritional properties. Hence, this biotechnology is also likely to ensure conservation of our environments. However, before PSM can contribute to such benefits, scientists must learn more about them and explore ways and means for their better utilization in the farmers' fields.

Future research should focus on managing plant–microbe interactions, particularly with respect to their mode of actions and adaptability to conditions under extreme environments for the benefit of plants. Furthermore, its need to address certain issues, like how to improve the efficacy of biofertilizers, what should be an ideal and universal delivery system, how to stabilize these microbes in soil systems, and how nutritional and root exudation aspects could be controlled in order to get maximum benefits from PSM application. Biotechnological and molecular approaches could possibly develop more understanding about PSM mode of actions that could lead to more successful plant-microbe interaction. Efforts should also be directed towards the use of PSM to reduce pesticide applications. In brief, PSM provides an excellent opportunity to develop environment-friendly phosphorus biofertilizer to be used as supplements and/or alternatives to chemical fertilizers.

Conclusion :

Phosphorus is a vital element in crop nutrition. Adverse environmental effects of chemical P fertilsers, depleting resources of high grade phosphatic rocks and their skyrocketing prices have compelled us to find a sustainable approach for efficient P availability in agriculture to meet the ever increasing demand of food. 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. In particular, microorganisms can solubilize and mineralize P from inorganic and organic pools of soil P.

The use of efficient phosphate-solubilizing microorganisms opens up a new horizon for better crop production besides sustaining soil health. However, the viability and sustainability of PSM largely depends on the development and distribution of good quality inoculants to farming communities. Therefore, it is a need for extensive and consistent research efforts to identify and characterize more PSM with greater efficiency for their ultimate application under different field conditions. Soil microbiologists have a great responsibility to find ways and means as to how soil available phosphorus content could be enhanced without applying the P fertilizers. It is clear that soil microorganisms play an important role in the mobilization of soil P and that detailed understanding of their contribution to the cycling of P in soil-plant systems is required for the development of sustainable agriculture and our movement from a green revolution to an evergreen revolution can be accomplished.

Authors' contributions :

Dr. Ajeet Kumar: Collected and reviewed the literature and prepared the manuscript. Dr. C.S. Choudhary: Provided guidance and improved the manuscript. Professor D. Paswan, Prof. B. Kumar and Smt. Anjana Arun have provided valuable suggestions in preparing the manuscript. All authors read and approved the final manuscript. Author Dr. Ajeet Kumar is the Principal Investigator and C.S. Choudhary is Co-PI of a project entitled "Study on native phosphate solubilizers of N-E alluvial plains of Bihar for development of efficacious phosphatic biofertilizers". The project is funded by the BAU, Sabour, Bhagalpur under Non-plan vide Project Code: S.P./NRM/RRS/2013-11. The insights gained through this review will help to understand the microbial diversity and their role in microbial transformation of various nutrients under the unique ecological zone of North-East Alluvial Plain of Bihar.

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