International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(3): 3905-3913
© 2019 IJCS
Received: 13-03-2019
Accepted: 15-04-2019

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Role of phosphorus solubilizing microorganisms and dissolution of insoluble phosphorus in soil

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Abstract

Phosphorus is the second important key element after nitrogen as a mineral nutrient in terms of quantitative plant requirement and most commonly limiting the growth of crops. Although abundant in soils, in both organic and inorganic forms, its availability is restricted as it occurs mostly in insoluble forms On average, the phosphorus content of soil is about 0.05% (w/w); however, only 0.1% of this phosphorus is available for plant use. The majority of the applied fertilizer phosphorus is not available to plants and the addition of inorganic fertilizers in excess of the amount that is commonly employed to overcome this effect can lead to environmental problems such as, groundwater contamination and waterway eutrophication. It is therefore of great interest to investigate management strategies that are capable of improving phosphorus fertilization efficiency, increase crop yields and reduce environmental pollution caused by phosphorus loss from the soil. Soil microorganisms enhance plant nutrient acquisition. They are involved in a wide range of biological processes including the transformation of insoluble soil nutrients. In the natural environment numerous microorganisms in the soil and rhizosphere are effective in releasing phosphorus from insoluble soil phosphorus through solubilization and mineralization. These groups of microorganisms are referred to as Phosphorus Solubilizing Microorganisms (PSM). Many species of soil fungi and bacteria are able to solubilize phosphorus *in vitro* and some of them can mobilize phosphorus in plants. PSM increase the bioavailability of soil insoluble phosphorus for plant use. They solubilize insoluble inorganic (mineral) phosphorus. Phosphate solubilizing/mineralizing microorganisms is therefore a promising strategy for the improvement of plant absorption of phosphorus and thereby reducing the use of chemical fertilizers that have a negative impact on the environment. Production of different types of organic acids by PSM and indirectly by other soil organisms in the main cause of phosphorus solubilization. Phosphorus solubilizers benefits cereals as well as legumes inoculation with Rhizobium.

Keywords: P-solubilizers, P solubilization, P-solubilization Mechanism

Introduction

Phosphorus (P) is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available Ezawa *et al*. 2002 [24]. Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition. Although microbial inoculants are in use for improving soil fertility during the last century, however, a meager work has been reported on P solubilization compared to nitrogen fixation. Soil P dynamics is characterized by physicochemical (sorption - desorption) and biological (immobilization-mineralization) processes. Large amount of P applied as fertilizer enters in to the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils Hao *et al*.2002. Organic matter is also an important reservoir of immobilized P that accounts for 20–80% of P in soils Richardson 1994). Only 0.1% of the total P exists in a soluble form available for plant up- take (*Zhou et al. 1992*) because of its fixation into an unavailable form due to P fixation.

Traditionally, the challenge of soil phosphorus deficiency is addressed by the application of phosphorus fertilizers. However, the majority of the applied fertilizer phosphorus is not available to plants and the addition of inorganic fertilizers in excess of the amount that is commonly employed to overcome this effect can lead to environmental problems such as, groundwater contamination and waterway eutrophication (Kang *et al*., 2011) [51]. It is therefore of great interest to investigate management strategies that are capable of improving phosphorus fertilization efficiency, increase crop yields and reduce environmental pollution caused by phosphorus loss from the soil.

Efficiency of P fertilizer throughout the world is around 10-25 % Isherword *et al.*1998 [39], and concentration of bio-available P in soil is very low reaching the level of 1.0 mg kg^{-1} soil Goldstein 2000 [29]. Soil microorganisms play a key role in soil P dynamics and subsequent availability of phosphate to plants Rishardson 2001. Inorganic forms of P are solubilized by a group of heterotrophic microrganisms excreting organic acids that dissolve phosphatic minerals and / or chelate cationic partners of the P ions directly, releasing P into solution He *et al*. 2002 [37]. Phosphate solubilizing bacteria (PSB) are being used as biofertilizer since 1950s Krasilinikov 1957 [48].

A large number of microbial organisms including bacteria, fungi, actinomycetes, and algae exhibit P solubilization ability. Many species of soil fungi and bacteria are able to solubilize phosphorus *in vitro* and some of them can mobilize phosphorus in plants. PSM increases the bioavailability of soil insoluble phosphorus for plant use. The inoculation of soil or crop with phosphate solubilizing microorganisms is therefore a promising strategy for the improvement of plant absorption of phosphorus. Thereby reducing the use of chemical fertilizers that have a negative impact on the environment. Soil bacteria that have been reported to mobilize poorly available phosphorus via solubilization include *Pseudomonas spp., Agrobacterium spp., and Bacillus circulans* (Babalola and Glick, 2012) [8].

Occurrence isolation of PSM

Solubilization of insoluble P by microorganisms was reported by Pikovskaya (1948) [67]. Several strains of bacterial and fungal species have been described and investigated in detail for their phosphate-solubilizing capabilities (Glick 1995; He *et al*. 1997) [28, 38]. Typically such microorganisms have been isolated using cultural procedures with species of Pseudomonas and Bacillus bacteria (Illmer and Schinner 1992) [42] and Aspergillus and Penicillium fungi being predominant (Wakelin *et al*. 2004) [92]. These organisms are ubiquitous but vary in density and mineral phosphate solubilizing (mps) ability from soil to soil or from one production system to another. In soil P solubilizing bacteria constitute 1-50% and fungi 0.1-0.5% of the total respective population. They are generally isolated from rhizosphere and nonrhi- zosphere soils, rhizoplane, phyllosphere, and rock P deposit area soil and even from stressed soils using serial plate dilution method or by enrichment culture technique (Zaidi *et al*. 2009). The concentration of iron ore, temperature, and C and N sources greatly influence the P-solubilizing potentials of these microbes. Among the various nutrients used by these microorganisms, ammonium salts has been found to be the best N source followed by asparagine, sodium nitrate, potassium nitrate, urea and calcium nitrate (Ahuja *et al*. 2007) [1]. Since 1948, when Pikovskaya suggested that microbes could dissolve non-readily available forms of soil P and play an important role in providing P to plants, numerous methods and media, such as Pikovskaya (Pikovskaya 1948) [67], bromophenol blue dye method (Gupta *et al*. 1994) [33] and National Botanical Research Institute P (NBRIP) medium (Nautiyal 1999) [61] 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 in true sense. Commonly used selection factor for this trait, tricalcium phosphate (TCP), is relatively weak and unreliable as a universal selection factor for isolating and testing phosphate solubilizing microorganisms (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 true PSM. Other compounds are also tested, but on a very small scale. These phosphates, mainly iron/aluminium phosphate and several calcium phosphates are even less soluble than TCP in water. Because soils greatly vary in pH and several chemical properties, it appears that there is no metal-Phosphate compound that can serve as the universal selection factor for PSM. Here multiple sources of insoluble phosphate are recommended. The selection of the metal-Phosphate candidates for potential PSM will depend on the type of soil (alkaline, acidic, or organic-rich) where the PSM will be used. Both bacterial and fungal strains exhibiting P solubilizing activity are detected by the formation of clear halo (a sign of solubilization) around their colonies. Production of a halo on a solid agar medium should not be considered the sole test for P solubilization. When colonies grow with- out a halo after several replacements of the medium, an additional test in liquid media to assay P dissolution 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 model plant as the ultimate test for potential P solubilization (Bashan *et al*. 2013) [10]. 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 biofertiliser.

Once a potential isolate is identified, it must be further tested for direct contribution to P plant nutrition and not necessarily to general growth promotion, as commonly done because promotion of growth by PSB, can be the outcome of other mechanisms. (Bashan *et al*. 2013a) and abilty to solubilise P is not necessarily correlated with the ability to promote plant growth (Collavino *et al*. 2010) [16]. The production of biofertilizer and its acceptance by farming communities are closely linked. For uptake by farmers, quality management is essential and must be performed consistently in order to supply reliable and contaminant-free bio products. As far as *in vitro* field trials are concerned the establishment and performance of these PSM inoculate developed in laboratory is largely hampered by environmental variables including salinity, pH, moisture, temperature and climatic conditions of the soil. Moreover it is also known that inocula developed from a particular soil fail to function as effectively in soils having different properties (Rodriguez and Fraga 1999) [76]. Hence there is a need to study PSM activity in correlation with these factors before PSM application as a biofertiliser.

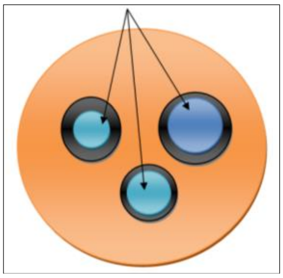


Fig 1: Clear zone formed around colony growth

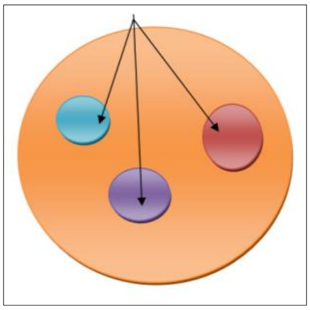


Fig 2: No Holo Zone

Isolation of PSM

Soil sample collection
↓
Serial dilution
↓
Inoculation of media (Pour plate/ Streaking)
↓
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 cultures by re inoculation
↓
Study of the morphology and colony characteristics 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
↓
Standarization (Quality control)

Biodiversity of P solubilizers

A substantial number of microbial species exhibit P solubilization capacity; these include bacteria, fungi, actino- mycetes and even algae. In addition to *Pseudomonas* and *Bacillus*, other bacteria reported as P-solubilizers include *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, *Delftia sp.* (Wani *et al*. 2005; Chen *et al*. 2006) [93, 14], *Azotobacter* (Kumar *et al*. 2001) [57], *Xanthomonas* (De Freitas *et al*. 1997) [18], *Enterobacter*, *Pantoea*, *and Klebsiella* (Chung *et al*. 2005) [15], *Vibrio proteolyticus*, *Xanthobacter agilis* (Vazquez *et al*. 2000) [91]. Furthermore, symbiotic nitrogenous *rhizobia*, which fix atmospheric nitrogen into ammonia and export the fixed nitrogen to the host plants, have also shown PS activity (Zaidi *et al*. 2009) For instance, *Rhizobium leguminosarum bv. Trifolii* (Abril *et al*. 2007) [2], and *Rhizobium* species nodulating *Crotalaria* species (Sridevi *et al*. 2007) [82] improved plant P-nutrition by mobilizing inorganic and organic P. Various PS bacteria have also been isolated from stressed environ- ments for example the halophilic bacteria *Kushneria sinocarni* isolated from the sediment of Daqiao saltern on the eastern coast of China, which may be useful in salt affected agricultural soils (Zhu *et al*. 2011) [98]. In soil, P-solubilizing fungi constitute about 0.1–0.5% of total fungal populations (Kucey 1983) [56].

P-solubilizing fungi do not lose the P dissolving activity upon repeated sub culturing under laboratory conditions as occurs with the P-solubilizing bacteria (Sperber 1958a, b; Kucey 1983) [83-84, 56]. Moreover, fungi in soils are able to traverse long distances more easily than bacteria and hence, may be more important to P solubilization in soils (Kucey 1983) [56]. Generally, the P-solubilizing fungi produce more acids than bacteria and consequently exhibit greater P-solubilizing activity (Venkateswarlu *et al*. 1984) [89]. Among filamentous fungi that solubilize phosphate, the genera *Aspergillus* and *Penicillium* (Fenice *et al*. 2000; Khan and Khan 2002; Reyes *et al*. 1999, 2002) [25, 49, 69, 70] are the most representative although strains of *Trichoderma* (Altomare *et al*. 1999) [3] and *Rhizoctonia solani* (Jacobs *et al*. 2002) [43] have also been reported as P solubilizers. A nematofungus *Arthrobotrys oligospora also* has the ability to solubilize phosphate *in vivo* as well as *in vitro* (Duponnois *et al.* 2006) [21]. 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) [88], *Schizosaccharomyces pombe* and *Pichiafermentans*. As more studies are conducted, a wider diversity of phosphate-solubilizing filamentous fungi are 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% after inoculation (Gunes *et al*. 2009) [32]. 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 (e.g. drought, fire.) but also possess other potential benefits (e.g. production of antibiotics and phytohormone-like compounds) that could simultaneously benefit plant growth (Fabre *et al*. 1988; Hamdali *et al*. 2008a, b) [23, 34, 36]. A study by Hamdali *et al*. (2008a) [34] has indicated that approximately 20% of actinomycetes can solubilize P, including those in the common genera *Streptomyces* and *Micromonospora*.

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 inter- action of AMF and the selected rhizobacteria has a po- tential to be developed as biofertilizers in acid soil. The potential of dual inoculation with AMF and rhizobac- teria needs to be further evaluated under different crop and agroclimatic conditions, particularly in the field (Widada *et al*. 2007).

Table 1: Biodiversity of PSM

Bacteria	*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,*
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.,*
Actinomycetes	*Actinomyces,, Streptomyces.*
Cyanobacteria	*Anabena sp., Calothrix braunii, Nostoc sp., Scytonema sp.,*
VAM	*Glomus fasciculatum.*

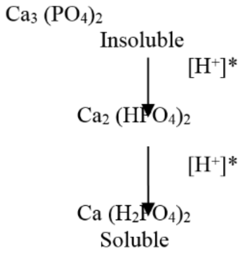
Mechanism of Phosphorus solubilization

The main 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) [59]. Therefore, microorganisms play an important role in all three major components of the soil P cycle (i.e. dissolution– precipitation, sorption–desorption, and mineralization– immobilization). Additionally these microorganisms in the presence of labile C serve as a sink for P, by rapidly immobilizing it even in low P soils; therefore PSM become a source of P to plants upon its release from their cells. 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) [11].

Inorganic P solubilization

by P-solubilizing microorganisms occurs mainly by organic acid production either by: (i) lowering the pH, or (ii) by enhancing chelation of the cations bound to P (iii) by competing with P for adsorption sites on the soil (iv) 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 (Whitelaw 2000; Maliha *et al*. 2004) [58] 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, mostly by oxidative respiration or by fermentation of organic carbon sources (e.g., glucose) (Atlas and Bartha 1997; Trolove *et al*. 2003) [5, 87] or 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 (Omar 1998) [64].

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 environment increases the divalent and trivalent forms of Pi (HPO^{-2} and HPO^{-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) [30]. Phosphate solubilising microorganisms produce various kinds of organic and inorganic acids which are cause of solubilisation by lowering the pH.



The prominent acids released by PSM in the solubilization of insoluble P are gluconic acid (Di-Simine *et al*. 1998; Bar-Yosef *et al*. 1999) [20, 9], oxalic acid, citricacid (Kim *et al*. 1997) [53], lactic acid, tartaric acid, aspartic acid (Venkateswarlu *et al*. 1984) [89]. Evidence from an abiotic study using HCl and gluconic acid to solubilize P also indicated that chelation of Al^{3+} by gluconic acid may have been a factor in the solubilization of colloidal Al phosphate (Whitelaw *et al*. 1999) [94]. Organic acids produced by P-solubilizing microorganisms can be detected by high performance liquid chromatography and enzymatic methods (Parks *et al*. 1990; 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) [85]. Altomare *et al*. (1999) [3] investigated the capability of the plant-growth promoting and biocontrol fungus *T. harzianum T-22* 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. 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 hydroxyapatite than citric acid or oxalic acid at same pH (Kim *et al*. 1997) [53]. H_2S produced by sulphate reducing bacteria of the genus *Desulfovibrio*.

$$\mathbf{FePO_4 + H_2S \rightarrow FeS + H_2PO_4^-}$$

Ferric phosphate Ferrous sulphide Available phosphate

Bacteria of the genera *Nitrosomonas* and *Thiobacillus* species can also dissolve phosphate compounds by producing nitric and sulphuric acids (Azam and Memon 1996) [6].

Nitrifying bacteria Form NO_3^-

$Ca_3(PO_4)_2 + 2\ HNO_3 \rightarrow 2\ CaHPO_4 + Ca(NO_3)_2$

$Ca_3(PO_4)_2 + 4\ HNO_3 \rightarrow Ca(H_2PO_4)_2 + 2Ca(NO_3)_2$

Sulphur oxidizing bacteria Form SO_2

$Ca_3(PO_4)_2 + H_2SO_4 \rightarrow CaHPO_4 + CaSO_4$

$Ca_3(PO_4)_2 + 2H_2SO_4 \rightarrow Ca(H_2PO_4)_2 + 2CaSO_4$

According to the sink theory, P-solubilizing organ- isms remove and assimilate P from the liquid and hence, activate the indirect dissolution of calcium phos- phate compounds by consistent removal of P from liquid culture medium. For instance, the P content in the biomass of *Pseudomonas* sp. and *P. aurantiogriseum* were similar to those observed in non-P-solubilizing microorganisms (Illmer *et al*. 1995) [41] which can be explained by the fact that the P content in biomass of organisms is consistently correlated with the decomposition of P containing organic substrates (Dighton and Boddy 1989) [19].

MPS activity occurs as a con- sequence ofmicrobial sulphur oxidation (Rudolph 1922) [78], nitrate production and CO_2 formation. These processes result in the formation of inorganic acids like sulphuric acid (Sperber 1958a) [83]. However, their effectiveness has been less accepted than the concept of involvement of organic acids in solubilization (Kim *et al*. 1997) [53]. H^+ excretion originating from NH^+ 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) [40]. They also reported that the most probable reason for solubilization without acid production is the release of protons accompanying respiration or NH^+ assimilation. Krishnaraj *et al*. (1998) [55] 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^+ driven pro- ton release seems to be the sole mechanism to promote P solubilization. Asea *et al*. (1988) [4] tested two fungi, *Penicillium bilaii* and *Penicillium fuscum*, for their ability to solubilize phosphate rock in the presence of NH^+ 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 (e.g. glucose versus fructose) rather than N supply (e.g. NH_4^+ versus NO_3^-) had the greatest effect on proton release (Park *et al*. 2009) [65]. 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) [69]. Acidification of the rhizosphere of cactus seedlings (giant cardon, *Pachycereus pringlei*) after inoculation with the plant growth-promoting bacterium *Azospirillum brasilense*, in the presence or absence of ammonium and nitrate, was studied and it was assumed that the effect of inoculation with this PGPB on plant growth, combined with nitrogen nutrition, might be affecting one or more of the metabolic pathways of the plant which increases proton efflux from roots and liberation of organic acid, leading to rhizosphere acidification (Carrillo *et al*. 2002). This indicates that for different species, different mechanisms are responsible for proton release, only partly depending on the presence of NH^+. Goldstein (1995) [31] 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.

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% of the total soil P (Khan *et al*. 2009b) [50]. Such P can be released from organic compounds in soil by enzymes:

i) 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 (often just called phosphatases) are the most abundant and best studied (Nannipieri *et al*. 2011) [60]. Depending on their pH optima, these enzymes are divided into acid and alkaline phosphomonoesterases and both can be produced by PSM depending upon the external conditions (Kim *et al*. 1998; Jorquera *et al*. 2008) [54, 44]. Typically, acid phosphatases predominate in acid soils, whereas alkaline phosphatases are more abundant in neutral and alkaline soils (Eivazi and Tabatabai 1977; Juma and Tabatabai 1977, 1998; Renella *et al*. 2006) [22, 45, 46, 68]. Although plant roots can produce acid phosphatases they rarely produce large quantities of alkaline phosphatases, suggesting that this is a potential niche for PSM (Juma and Tabatabai 1998; Criquet *et al*. 2004) [46, 17]. It is also difficult to differentiate between root- and PSM-produced phosphatases (Richardson *et al*. 2009a, b) [74-75] 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) [86]. The relationship between PSM introduced into soil, phosphatase activity and the subsequent mineralization of Po still remains poorly understood (Chen *et al*. 2003) [13].

ii) Phytases, which specifically cause release of P from phytate degradation. In its basic form, phytate is the primary source of inositol and the major stored form of P in plant seeds and pollen, and is a major component of organic P in soil (Richardson, 1994) [71]. 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 (phyA) derived from *Aspergillus niger* (Richardson *et al*. 2001) [72]. 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 in fact a 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) [73].

iii) Phosphonatases and C–P lyases, that cleave the C–P bond of organophosphonates (Rodriguez *et al*. 2006) [77].

It is therefore clear that P solubilization by PSMs has been a subject of analysis and research for a long time and still the research seems to be in its infancy. It occurs through different mechanisms and there is considerable variation amongst the organisms in this respect. Each organism can act in one or more than one way to bring about the solubilization of insoluble P. Though it is difficult to pin point a single mechanism, production of organic acids and consequent pH reduction appears to be of great importance. Different mechanisms involved in the solubilization and mineralization of insoluble P by naturally-occurring microbial communities of soils.

Factors Influencing Microbial Phosphate Solubilization

The ability of PSM to transform insoluble organic and inorganic phosphorus is associated with, the nutritional richness of the soil, and the physiological and growth status of the organism. PSM from soils from environmental extremes such as saline-alkaline soils, soil with a high level of nutrient deficiency, or soil from extreme temperature environments have the tendency to solubilize more phosphate than PSM from soils from more moderate conditions (Zhu *et al*., 2011) [98]. There has been a conflicting report on the influence of temperature on phosphorus solubilization by microbes. White *et al*. (1997) [95] found 20–25°C as the optimum temperature for maximum microbial phosphorus solubiliztion while 28°C was reported by Kang *et al*. (2002) [52], and Varsha (2002) [90]. In addition, others including Kim *et al*. (1997) [53], Rosado *et al*. (1998) [79], Johri *et al*. (1999) [47], and Fasim *et al*. (2002) [27], have recorded 30°C as the best temperature for P solubilization. Nahas (1996) [2] and Nautiyal *et al*. (2000) [63] reported P solubilization at extreme temperature of 45°C in desert soil while Johri *et al*. (1999) [47] reported solubilization at a low temperature of 10°C.

Among other factors influencing microbial phosphate solubilization are interactions with other microorganisms in the soil, the extent of vegetation, ecological conditions, climatic zone soil types, plant types, agronomic practices, land use systems, and the soil's physicochemical properties such as organic matter and soil pH (Seshachala and Tallapragada, 2012) [80]. Phosphorus is solubilized faster in warm humid climates and slower in cool dry climates. A well-aerated soil will more readily permit rapid phosphorus solubilisation compared to a saturated wet soil. The land use system is the use that the farmland has been previously committed to, such as cropping or livestock activities or even mixed use. Recently, Zhang *et al*. (2014) [96] reported that adding small amounts of inorganic phosphorus to the rhizosphere could drive phytic acid mineralization by bacteria and thereby improve plant phosphorus nutrition. Lime and compost, used as a soil improver, also had positive effects on phosphate solubilizers. Phosphorus Solubilizing Bacteria population richness and diversity, according to Azziz *et al*. (2012) [7], were more abundant and diverse following crop rotation. Soil rich in organic matter will favor microbial growth and therefore favors microbial phosphorus solubilisation. Soil pH values between 6 and 7.5 are best for P-availability, this is because at pH values below 5.5 and between 7.5 and 8.5 limits P from becoming fixed by aluminum, iron, or calcium, and hence, not being available for plant use. A negative correlation was observed between the amount of phosphate solubilized by *B. cepacia* SCAUK0330 and the pH drop that is associated with this process. The pH drop leads to an increase in phosphate solubilization. At pH 3.12, 452 $\mu g \cdot mL^{-1}$ of phosphorus was solubilized, and when 154 $\mu g \cdot mL^{-1}$ of P was solubilized the pH value was 4.95 (Zhao *et al*., 2014) [97]. Research has also shown that microbial phosphate solubilization largely depends on the kinds of metabolite produced and its rate of release (Zhu *et al*., 2011) [98].

Benefits of Phosphorus Solubilizing Microorganism

i) For better utilization of the phosphorus accumulated in soils, PSMs that are capable of transforming insoluble phosphorus to soluble forms can function as biofertilizers. This increases the soluble phosphorus content (Zhu *et al*., 2012) [99].
ii) The use of phosphorus biofertilizers is a promising approach to improving food production through enhancing agricultural yield as it is better to use an environmentally friendly approach to solve the problems of infertile soil (Babalola and Glick, 2012) [12].

Future Prospects

As additional insights are gained regarding PSM and the mechanisms that they use, there is every reason to believe that the use of PSM as biofertilizers will likely improve their use, as effective and important components in the establishment of sustainable soil management systems. The focus of consumers of agricultural produce is on the health, quality and nutritional value of those products. Thus, the employment of PSM as biofertilizers is an option that can increase food production without imposing any health hazard, and at the same time conserve the environment. It is essential that researchers continue to learn more about PSM and, immediately, translate this knowledge into a form that can readily be used by farmers.

Conclusion

This review has shown that phosphate-solubilizing microorganisms have tremendous potential as Bio-fertilizers. Mobilizing soil inorganic phosphate and increasing its bioavailability for plant use by harnessing soil PSM promotes sustainable agriculture, improves the fertility of the soil, and hence increases crop productivity. The use of PSM as microbial inoculants is a new horizon for better plant productivity. PSM technology can contribute to low-input farming systems and a cleaner environment. However, there is need to develop PSB technologies specific to various regions and this should be communicated to farmers in a relatively short time.

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