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REVIEW

Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils

Seema B Sharma[1*], Riyaz Z Sayyed[2], Mrugesh H Trivedi[1] and Thivakaran A Gobi[3]

Abstract

Phosphorus is the second important key element after nitrogen as a mineral nutrient in terms of quantitative plant requirement. Although abundant in soils, in both organic and inorganic forms, its availability is restricted as it occurs mostly in insoluble forms. The P content in average soil is about 0.05% (w/w) but only 0.1% of the total P is available to plant because of poor solubility and its fixation in soil (Illmer and Schinner, Soil Biol Biochem 27:257-263, 1995). An adequate supply of phosphorus during early phases of plant development is important for laying down the primordia of plant reproductive parts. It plays significant role in increasing root ramification and strength thereby imparting vitality and disease resistance capacity to plant. It also helps in seed formation and in early maturation of crops like cereals and legumes. Poor availability or deficiency of phosphorus (P) markedly reduces plant size and growth. Phosphorus accounts about 0.2 - 0.8% of the plant dry weight.

To satisfy crop nutritional requirements, P is usually added to soil as chemical P fertilizer, however synthesis of chemical P 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 can use only a small amount of this 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 of P nutrition of crops. In this regards phosphate-solubilizing microorganisms (PSM) have been seen as best eco-friendly means for P nutrition of crop. Although, several bacterial (*pseudomonads and bacilli*) and fungal strains (*Aspergilli* and *Penicillium*) have been identified as PSM 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. This review focuses on the diversity of PSM, mechanism of P solubilization, role of various phosphatases, impact of various factors on P solubilization, the present and future scenario of their use and potential for application of this knowledge in managing a sustainable environmental system.

Keywords: Soil phosphorus; PSM; P solubilization; Biodiversity; Biofertilizers

Introduction

Phosphorus is the most important key element in the nutrition of plants, next to nitrogen (N). It plays an important role in virtually all major 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, it is a major limiting factor for plant growth as it is in an unavailable form for root uptake. Inorganic P occurs in soil, mostly in insoluble mineral complexes, some of them appearing after frequent application of chemical fertilizers. These insoluble, precipitated forms cannot be absorbed by plants (Rengel and Marschner 2005). 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 uptake (Zhou et al. 1992) because of its fixation into an unavailable form due to P fixation. The term P fixation is

* Correspondence: seemabhargavsharma@gmail.com
[1]Department of Earth and Environmental Science, KSKV Kachchh University, Mundra Road, Bhuj 370 001, Gujarat, India
Full list of author information is available at the end of the article

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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). The soils that exhibit highest P fixation capacity occupy 1,018 million hectares (ha) in the tropics (Sanchez and Logan 1992). It is for this reason that soil P becomes fixed and available P levels have to be supplemented on most agricultural soils by adding chemical P fertilizers, which not only represent a major cost of agricultural production but also impose adverse environmental impacts on overall soil health and degradation of terrestrial, freshwater and marine resources (Tilman et al. 2001). Thus, increased P levels have been identified as a main factor for eutrophication of surface waters that may lead to algal blooms (Schindler et al. 2008). 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 phosphate fertilizers on microbial activities includes inhibition of substrate-induced respiration by streptomycin sulphate (fungal activity) and actidione (bacterial activity) and microbial biomass carbon (C) (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).

Moreover the efficiency of applied P fertilizers in chemical form rarely exceeds 30% 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 P in agricultural soils would be sufficient to sustain maximum crop yields worldwide for about 100 years if it were available (Khan et al. 2009a, b, c). A major characteristic of P biogeochemistry is that only 1% of the total soil P (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; Quiquampoix and Mousain 2005). Furthermore P is a finite resource and based on its current rate of use, it has been estimated that the worlds known reserves of high quality rock P may be depleted within the current century (Cordell et al. 2009). Beyond this time the production of P based fertilizers will require the processing of lower grade rock at significantly higher cost (Isherwood 2000). The realization of all these potential problems associated with chemical P fertilizers together with the enormous cost involved in their manufacture, has led to the search for environmental compatible and economically feasible alternative strategies for improving crop

production in low or P-deficient soils (Zaidi et al. 2009). The use of microbial inoculants (biofertilisers) possesing P-solubilizing activities in agricultural soils is considered as an environmental-friendly alternative to further applications of chemical based P fertilizers.

Constraints in using phosphate fertilizers

There is global concern about the energy and costs involved in mining the rock phosphate and its transport from manufacturing sites to farm crop fields. Mining phosphate minerals and spreading P fertilizers over the landscape is neither eco-friendly, economically feasible nor it is sustainable and it poses following constraints (i) emission of the fluorine as the highly volatile and poisonous HF gas, (ii) disposal of gypsum and (iii) accumulation of Cd and other heavy metals in soil and possibly crops as a result of repetitive use of P fertilizers.

At present mining rate (about 7,100 million tones/annum), reserve will be depleted in about 500–600 years. In India, deposits of sufficiently enriched phosphatic rocks are limited and hence it imports 2 million tons of rock phosphate annually. About 98% of cropland in India is deficient in available forms of soil phosphorus and only 1-9% has high phosphorus status. Intensive cropping pattern during this green and white revolution has also resulted in widespread deficiency of phosphorus. Although various amendments are available for management of P in different soil, all are costlier and practically difficult. Thus, even if the total soil P is high and also if P fertilizers are applied regularly, pH dependent chemical fixation determines the quantity of available P. The holistic P management involves a series of strategies involving manipulation of soil and rhizosphere processes, development of P efficient crops and improving P recycling efficiency. Microbial mediated P management is an ecofriedly and cost effective approach for sustainable development of agricultural crop.

Microorganisms are an integral component of the soil P cycle and are important for the transfer of P between different pools of soil P. Phosphate Solubilzing Microorganisms (PSM) through various mechanisms of solubilization and mineralisation are able to convert inorganic and organic soil P respectively (Khan et al. 2009a) into the bioavailable form facilitating uptake by plant roots. It is important to determine the actual mechanism of P solubilisation by PSM for optimal utilization of these microorganisms in varied field conditions. Hence it is imperative to better understand the plant-soil-microbial P cycle with the aim of reducing reliance on chemical P fertilizers. This has led to increased interest in the harnessing of microorganisms to support P cycling in agroecosystems.

Occurrence and isolation of PSM

Solubilization of insoluble P by microorganisms was reported by Pikovskaya (1948). During the last two decades

knowledge on phosphate solubilizing microorganisms increased significantly (Richardson 2001; Rodriguez and Fraga 1999). 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). 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). 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 nonrhizosphere 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). 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), bromophenol blue dye method (Gupta et al. 1994) and National Botanical Research Institute P (NBRIP) 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 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 (up to several thousand per study) 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. 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 Bashan et al. 2013a, b.
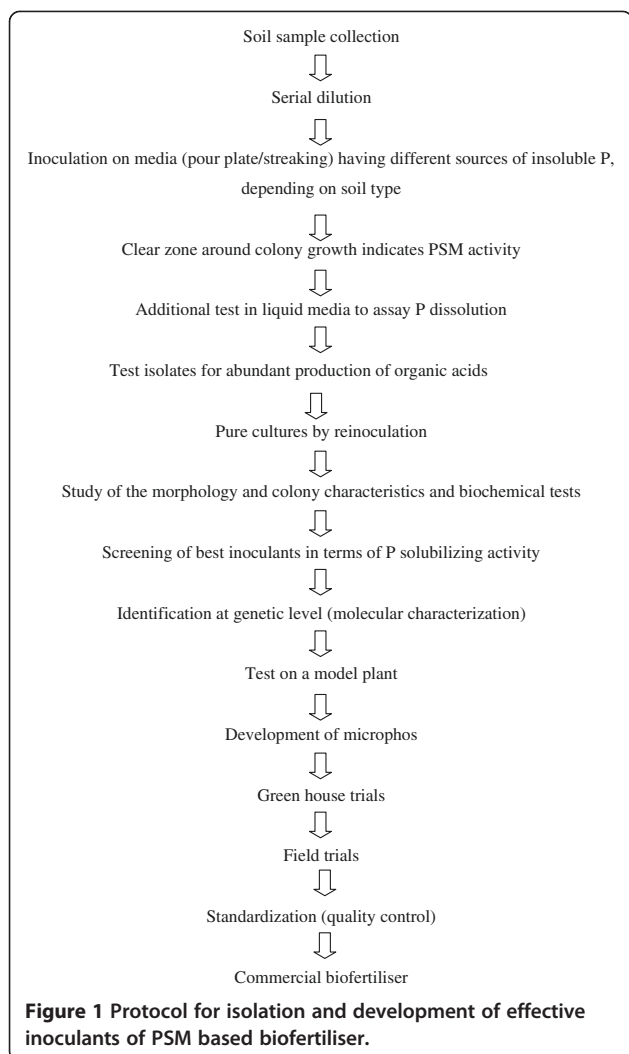
Both bacterial and fungal strains exhibiting P solubilizing activity are detected by the formation of clear halo (a sign of solubilization) around their colonies. Production of a halo on a solid agar medium should not be considered the sole test for P solubilization. When colonies grow without a halo 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. 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 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, even by PSB, can be the outcome of other mechanisms. (Bashan et al. 2013a) and ablity to solubilise P is not necessarily correlated with the ability to promote plant growth (Collavino et al. 2010).

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). Hence there is a need to study PSM activity in correlation with these factors before PSM application as a biofertiliser. Protocol for isolation and effective inoculants development of PSM based biofertiliser has been shown in Figure 1.

Biodiversity of P solubilizers

A substantial 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

Soil sample collection

⇓

Serial dilution

⇓

Inoculation on media (pour plate/streaking) having different sources 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 cultures by reinoculation

⇓

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

⇓

Standardization (quality control)

⇓

Commercial biofertiliser

Figure 1 Protocol for isolation and development of effective inoculants of PSM based biofertiliser.

Rhodococcus, Arthrobacter, Serratia, Chryseobacterium, Gordonia, Phyllobacterium, Delftia sp. (Wani et al. 2005; 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 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), and *Rhizobium* species nodulating *Crotalaria* species (Sridevi et al. 2007) improved plant P-nutrition by mobilizing inorganic and organic P. Various PS bacteria have also been isolated from stressed environments 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).

In soil, P-solubilizing fungi constitute about 0.1–0.5% 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 (Sperber 1958a, b; Kucey 1983). Moreover, fungi in soils are able to traverse long distances more easily than bacteria and hence, may be more important to P solubilization in soils (Kucey 1983). Generally, the P-solubilizing fungi produce more acids than bacteria and consequently exhibit greater P-solubilizing activity (Venkateswarlu et al. 1984). Among filamentous fungi that solubilize phosphate, the genera *Aspergillus* and *Penicillium* (Fenice et al. 2000; Khan and Khan 2002; Reyes et al. 1999, 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 nematofungus *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 *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).

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). A study by Hamdali et al. (2008a) 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.

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 (Widada et al. 2007).

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.*

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 from varied environments in coming times.

Mechanism of P-solubilization by PSM

In a review of P chemistry in soils, Sims and Pierzynski (2005) identified the major processes of the soil P cycle that affect soil solution P concentrations as(1) dissolution–precipitation (mineral equilibria), (2) sorption–desorption (interactions between P in solution and soil solid surfaces), and (3) mineralization–immobilization (biologically mediated conversions of P between inorganic and organic forms).

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). 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). (Grierson et al. 1998) found that about 30–45% 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.

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

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_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 (Di-Simine et al. 1998; Bar-Yosef et al. 1999), oxalic acid, citricacid (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 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

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

Organism	Ecological niche	Predominant acids produced	Reference
PSB	Soil and phosphate bearing rocks	ND(not determined)	Pikovskaya 1948
PSB	Bulk and rhizospheric soil	ND(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
Bacillus amyloliquefaciens, B. licheniformis, B. atrophaeus, Penibacillus macerans, Vibrio proteolyticus, xanthobacter agilis, Enterobacter aerogenes, E. taylorae, E. asburiae, Kluyvera cryocrescens, Pseudomonas aerogenes, Chryseomonas Luteola	Mangrove ecosystem	Lactic, itaconic, isovaleric, isobutyric, acetic	Vazquez et al. 2000
Penicillium rugulosum	Venezuelan phosphate rocks	Citric, gluconic acid	Reyes et al. 2001
Enterobacter intermedium	Grass rhizosphere	2-ketogluconic	Hwangbo et al. 2003
Aspergillus flavus, A . niger, Penicilliumcanescens	stored wheat grains	Oxalic, citric, gluconic succinic	Maliha et al. 2004
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
P.trivialis	rhizosphere of *Hippophaer hamnoides* growing in the cold deserts of Lahaul and Spiti in the trans-Himalayas	Lactic, formic	Vyas and Gulati 2009
B.pumilus var.2;B.subtilisvar.2;Actinomadura oligospora; Citrobacter sp.	Giant Cardon cactus (P.pringlei) growing in ancient lava	Gluconic, Propionic, Isovaleric, Heptonic, Caproic, Isocaproic, Formic, Valeric, Succinic, Oxalic, Oxalacetic, Malonic.	Puente et al. 2004a
B.pumilus CHOO8A; B.fusiformis	Cholla cactus(Opuntia Cholla)		Puente et al. 2004a
Bacillus sp. SENDO 6 and	Giant Cardon cactus (P.pringlei)	Gluconic, Propionic, Isovaleric, Formic, Succinic, Lactic.	Puente et al. 2009a, b
Pseudomonas putida M5TSA, Enterobacter sakazakii M2PFe, and Bacillus megaterium M1PCa	Wild cactus Mammilaria fraileana		Lopez et al. 2011

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 (SubbaRao 1982). Altomare et al. (1999) 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. 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 hydroxyapatite 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 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) 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).

The other mechanism is the **production of H₂S,** which react with ferric phosphate to yield ferrous sulphate with concomitant release of phosphate (Swaby and Sperber 1958).

It has been suggested that MPS activity occurs as a consequence of **microbial sulphur oxidation** (Rudolph 1922), nitrate production and CO_2 formation. These processes result in the formation of inorganic acids like sulphuric acid (Sperber 1958a). However, their effectiveness has been less accepted than the concept of involvement of organic acids in solubilization (Kim et al. 1997).

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 (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). 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). 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_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* DA23.

B) 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). 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). 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). 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). 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). It is also difficult to differentiate between root- and PSM-produced phosphatases (Richardson et al. 2009a, 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)

(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). 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). 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).

(iii) phosphonatases and C–P lyases, that cleave the C–P bond of organophosphonates (Rodriguez et al. 2006).

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 are briefly illustrated in Figure 2.

Role of siderophores in P solubilization

Siderophores are complexing agents that have a high 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 approximately 500 known siderophores, with the majority of them being used by a wide range of microorganisms and plants and some of them being exclusively used by the microbial species and strains that produce them (Crowley 2007). Studies have reported the release of siderophores from PSM (Vassilev et al. 2006; Caballero-Mellado et al. 2007; Hamdali et al. 2008a); however, siderophore production 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 by Yi et al. (2008). 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
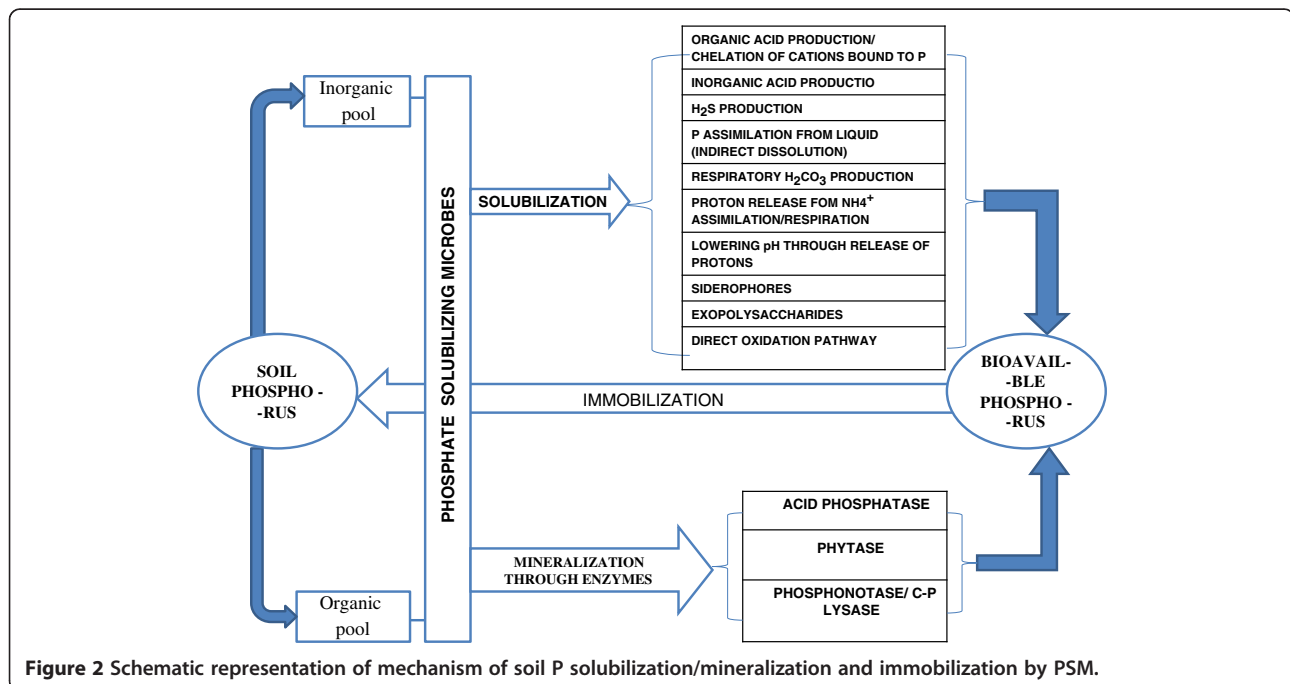

Figure 2 Schematic representation of mechanism of soil P solubilization/mineralization and immobilization by PSM.

solubilization of P by Yi et al. 2008. These Phosphate Solu-bilizing 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.

Plant growth promoting attributes of PSM

Besides making soluble P accessible for uptake by plants, there have been a number of reports on plant growth pro-motion by these microorganisms (Gaur and Ostwal 1972). This is achieved by production of plant beneficial metabo-lites, such as phytohormones, antibiotics, or siderophores. Various PSM preparations have been shown to promote the growth of many crops (Table 3). Endophytic Bacteria isolated from rhizoplane of cacti growing in bare lava rocks, not only significantly mobilized Phosphate and other minerals (Puente et al. 2004a, 2009a) but also promoted growth of wild cactus species (Puente et al. 2004b, b). The mechanisms involved in plant growth promotion by PSM are outlined in Figure 3.

Genetic engineering of PSM

Although knowledge of the genetics of phosphate solubilization is still scanty, and the studies at the molecu-lar level in order to understand how precisely the PSM brings out the solubilization of insoluble P are inconclu-sive (Rodriguez et al. 2006). However, some genes involved in mineral and organic phosphate solubilization have been isolated and characterized. Initial achievements in the manipulation of these genes through genetic engineering and molecular biotechnology followed by their expression in selected rhizobacterial strains open a promising perspec-tive for obtaining PSM strains with enhanced phosphate solubilizing capacity, and thus, a more effective use of these microbes as agricultural inoculants. The initial achievement in cloning of gene involved in P solubilization from the Gram negative bacteria *Erwinia herbicola* was achieved by Goldstein and Liu (1987). Similarly the napA phosphatase gene from the soil bacterium *Morganella morganii* was transferred to *Burkholderia cepacia IS-16*, a strain used as a biofertilizer, using the broad-host range vector pRK293 (Fraga et al. 2001). An increase in extracellular phosphatase activity of the recombinant strain was achieved.

## Table 3 Plant growth promotion by PSM (Patil et al. 2002)

PSM Bioinoculant	Crop benefited
B. firlmus NCIM 2636	Paddy in acid soils
G. faciculatum	Banana
B. megaterium+*G. faciculatum*	Banana
Phosphobacterium	Sword bean variety SBS 1
P. Striata	Soybean in sandy alluvial soil
P. Striata	Chick pea

Introduction or over-expression of genes involved in soil phosphate solubilization (both organic and inorganic) in natural rhizosphere bacteria is a very attractive approach for improving the capacity of microorganisms to work as inoculants. Insertion of phosphate-solubilizing genes into microorganisms that do not have this capability may avoid the current need of mixing two populations of bacteria, when used as inoculants (nitrogen fixers and phosphate-solubilizers (Bashan et al. 2000). There are several advantages of developing genetically-modified PSM over transgenic plants for improving plant performance: (1) With current technologies, it is far easier to modify a bacterium than complex higher organisms, (2) Several plant growth-promoting traits can be combined in a single organism, and (3) Instead of engineering crop by crop, a single, engineered inoculant can be used for several crops, especially when using a non-specifc genus like Azospirillum (Rodriguez et al. 2006). Some barriers should be overcome first to achieve successful gene inser-tions using this approach, such as the dissimilarity of metabolic machinery and different regulating mechanism between the donor and recipient strains. Despite the diff-culties, significant progress has been made in obtaining genetically engineered microorganisms for agricultural use (Armarger 2002). Overall, further studies on this aspect of PSM will provide crucial information in future for better use of these PSM in varied environmental conditions.

Current trends

Phosphorus is an important limiting factor in agriculture production, and considering the negative effects of chem-ical P fertilizers, microbial intervention of PSM seems to be an effective way to solve the phosphorus availability in soil. However P-solubilization in soil is much more difficult to study than solubilization of P 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 micro-organism used. Hence study of PSM activity in correlation with these factors has to be done extensively before PSM can be used as a biofertiliser with promising results. The successful implementation of this approach has already been demonstrated in the fields by various workers, to a limited extent. However the large scale use of this technol-ogy would benefit from additional studies, particularly those directed towards understanding how the interaction between soil and microbial system might be facilitated.

The organisms involved in phosphorus (P) cycling in soils are highly varied, and microorganisms probably play the most important role. However, more than 99% 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

Figure 3 Possible Mechanisms involved in plant growth promotion by PSM.
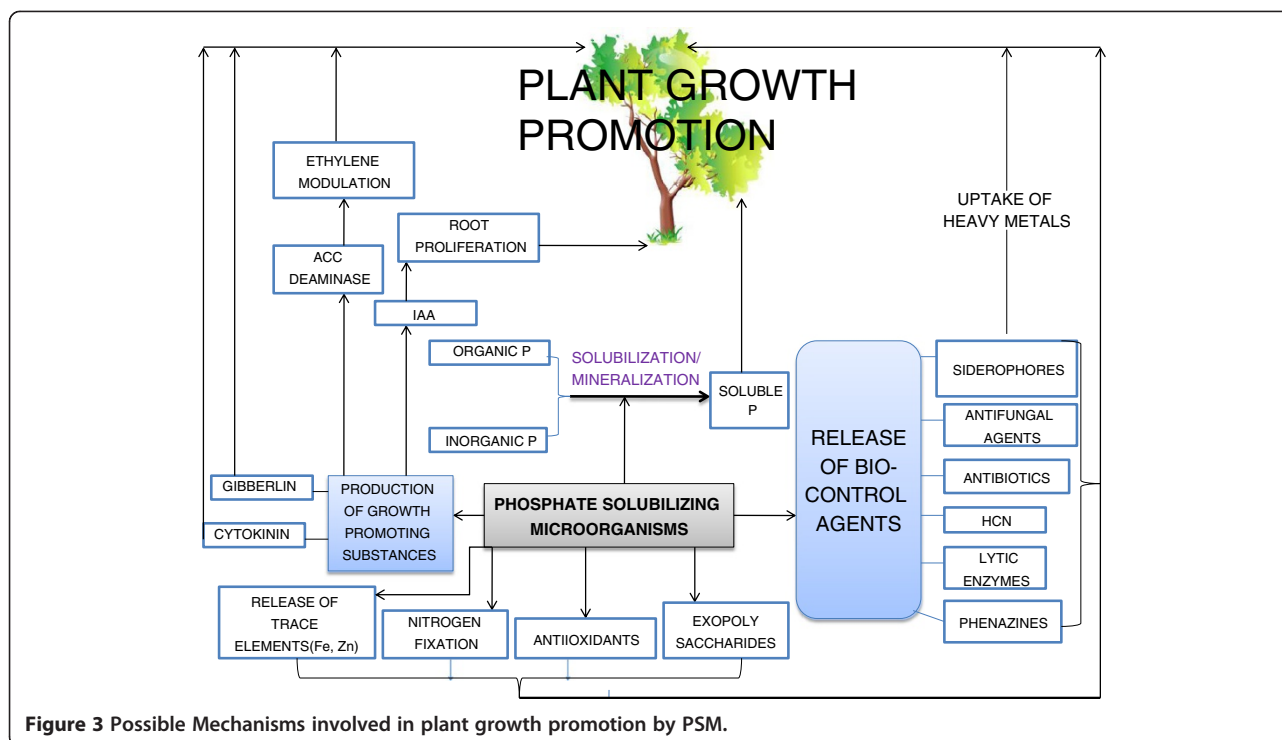
microbes involved in P cycling in soils. Molecular approaches for such culture-independent methods have been developed in the recent past. The molecular techniques based on nucleic acid composition like LMW RNA profiling and PCR based techniques, are excellent tools for this purpose, as they are precise, reproducible and not dependent on culture media composition or growth phase of microorganisms (Peix et al. 2007).

Molecular-based techniques also provide new opportunity to detect the presence and abundance of specific microorganisms or to quantify the expression of target genes directly in soil or in the rhizosphere with high levels of sensitivity. For example, specific primers based on conserved regions have been described for various microorganisms associated with P mobilization, including mycorrhizal fungi, Penicillium sp., and Pseudomonas sp. (Oliveira et al. 2009), as have primers that are directed at traits such as bacterial phytases (Jorquera et al. 2011). Microarrays composed of suites of functional bacterial genes and arrays for phylogenetic analysis of bacterial diversity based on 16S-RNAgene sequences along with next-generation sequencing and soil microbiome analyses; provide further application for assessment of diversity surrounding particular traits or functional groups of micro-organisms (Richardson and Simpson 2011). Collectively, these tools provide new opportunities to address key questions in microbial community ecology and to assess the survival and persistence of specific inoculants under different environmental conditions.

Looking at the possible avenues which can open up with exploring these environmental friendly microorganisms, it is necessary to study the composition and dynamics of these microbial populations to reach a better understanding of soil PSM diversity and P uptake by plants.

Future prospects
Despite their 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 sustainable 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 biofertilisers 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, more than ever, obtaining high yields is the main challenge for agriculture. In addition, 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, scientists 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 biotechnology provides an excellent opportunity to develop environment-friendly phosphorus biofertilizer to be used as supplements and/or alternatives to chemical fertilizers.

Conclusions

Phosphorus is a vital element in crop nutrition. Adverse environmental effects of chemical based 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 global 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 total soil P.

The use of efficient PSM (phosphate-solubilizing microorganisms), opens up a new horizon for better crop productivity besides sustaining soil health. However, the viability and sustainability of PSM technology largely depends on the development and distribution of good quality inoculants to farming communities. Therefore, there is a need for extensive and consistent research efforts to identify and characterize more PSM with greater efficiency for their ultimate application under field conditions. Soil Scientists and Microbiologists have a great responsibility to the society to find ways and means as to how soil P could be improved without applying the chemical P fertilizers under different agro-climatic regions of the world.

The promise of exploiting soil microorganisms to increase mobilization of soil P remains. Whether or not this will be achieved through better management of soil microbial communities, by development of more effective microbial inoculants, through the genetic manipulation of specific organisms, or with a combination of these approaches is not known. What is clear though is 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

SBS: Collected and reviewed the literature and drafted the manuscript. RZS: Provided guidance and improved the manuscript. MHT: provided guidance. GAT: provided guidance. All authors read and approved the final manuscript.

Authors' information

Author SBS is the Principal Investigator of a project entitled "Study of the microbiological diversity in different agricultural soils of kachchh with special reference to Phosphate Solubilising Microbes". The project is funded by the Women Scientist Scheme (WOS-A) of Department Of Science And Technology, Government of India, New Delhi. Under this project agricultural fields applying different amendments are studied for their microbial diversity as well as physico chemical properties in various seasons and any correlation which, if exists is being studied. The insights gained through this study will help to understand the microbial diversity in this very unique ecological zone of Kachchh, Gujarat, Western India.

Author details

[1]Department of Earth and Environmental Science, KSKV Kachchh University, Mundra Road, Bhuj 370 001, Gujarat, India. [2]Department of Microbiology, PSGVP Mandal's Arts, Science and Commerce College, Shahada 425409, Maharashtra, India. [3]Gujarat Institute of Desert Ecology, Bhuj, Gujarat, India.

References

Abril A, Zurdo-Pineiro JL, Peix A, Rivas R, Velazquez E (2007) Solubilization of phosphate by a strain of *Rhizobium leguminosarum* bv. *Trifolii* isolated from Phaseolus vulgaris in El Chaco Arido soil (Argentina). In: Velazquez E, Rodriguez-Berrueco C (eds) Developments in Plant and Soil Sciences. Springer, The Netherlands, pp 135–138

Ahuja A, Ghosh SB, D'Souza SF (2007) Isolation of a starch utilizing, phosphate solubilizing fungus on buffered medium and its characterization. Bioresour Technol 98:3408–3411

Altomare C, Norvell WA, Borjkman T, Harman GE (1999) Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus Trichoderma harzianum Rifai 1295–22. Appl Environ Microbiol 65:2926–2933

Armarger N (2002) Genetically modified bacteria in agriculture. Biochimie 84:1061–1072

Asea PEA, Kucey RMN, Stewart JWB (1988) Inorganic phosphate solubilization by two Penicillium species in solution culture and soil. Soil Biol Biochem 20:459–464

Atlas R, Bartha R (1997) Microbial ecology. Addison Wesley Longman, New York

Azam F, Memon GH (1996) Soil organisms. In: Bashir E, Bantel R (eds) Soil science. National Book Foundation, Islamabad, pp 200–232

Bajpai PD, Sundara Rao WVB (1971) Phosphate solubilizing bacteria III. Soil inoculation with phosphate solubilizing bacteria. Soil Sci Plant Nutr 17:46–53

Banik S, Dey BK (1983) Phosphate solubilizing potentiality of the microorganisms capable of utilizing aluminium phosphate as a sole phosphate source. Zentralblatt Microbiology 138:17–23

Barber SA (1995) Soil nutrient bioavailability. A mechanistic approach, Wiley, New York

Bar-Yosef B, Rogers RD, Wolfram JH, Richman E (1999) *Pseudomonas cepacia*-mediated rock phosphate solubilization in kaolinite and montmorillonite suspensions. Soil Sci Soc Am J 63:1703–1708

Bashan Y, Moreno M, Troyo E (2000) Growth promotion of the seawater-irrigated oil seed halophyte Salicornia bigelovii inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* sp. Biol Fertil Soils 32:265–272

Bashan Y, Kamnev AA, de Bashan LE (2013a) A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth. Biol Fertil Soils 49:1–2

Bashan Y, Kamnev AA, de Bashan LE (2013b) Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure. Biol Fertil Soils 49:465–479

Blake L, Mercik S, Koerschens M, Moskal S, Poulton PR, Goulding KWT, Weigel A, Powlson DS (2000) Phosphorus content in soil, uptake by plants and balance in three European long-term field experiments. Nutr Cycl Agroecosyst 56:263–275

Bolan NS, Currie LD, Baskaran S (1996) Assessment of the influence of phosphate fertilizers on the microbial activity of pasture soils. Biol Fertil Soils 21:284–292

Butterly CR, Bunemann EK, McNeill AM, Baldock JA, Marschner P (2009) Carbon pulses but not phosphorus pulses are related to decrease in microbial biomass during repeated drying and rewetting of soils. Soil Biol Biochem 41:1406–1416

Caballero-Mellado J, Onofre-Lemus J, De los Santos EP, Martinez-Aguilar L (2007) The tomato rhizosphere, an environment rich in nitrogen-fixing Burkholderia species with capabilities of interest for agriculture and bioremediation. Appl Environ Microbiol 73:5308–5319

Carrillo AE, Li CY, Bashan Y (2002) Increased acidification in the rhizosphere of cactus seedlings induced by *Azospirillum brasilense*. Naturwissenschaften 89:428–432

Chandini TM, Dennis P (2002) Microbial activity, nutrient dynamics and litter decomposition in a Canadian Rocky Mountain pine forest as affected by N and P fertilizers. For Ecol Manage 159:187–201

Chen CR, Condron LM, Davis MR, Sherlock RR (2003) Seasonal changes in soil phosphorus and associated microbial properties under adjacent grassland and forest in New Zealand. Forest Ecol Manag 117:539–557

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Chuang CC, Kuo YL, Chao CC, Chao WL (2007) Solubilization of inorganic phosphates and plant growth promotion by *Aspergillus niger*. Biol Fertil Soils 43:575–584

Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol Biochem 37:1970–1974

Collavino MM, Sansberro PA, Mroginski LA, Aguilar OM (2010) Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote Phaseolus vulgaris growth. Biol Fertil Soils 46:727–738

Cordell D, Drangert JO, White S (2009) The story of phosphorus: global food security and food for thought. Glob Environ Chang 19:292–305

Criquet S, Ferre E, Farner EM, Le Petit J (2004) Annual dynamics of phosphatase activities in an evergreen oak litter – influence of biotic and abiotic factors. Soil Biol Biochem 36:1111–1118

Crowley DE (2007) Microbial siderophores in the plant rhizosphere. In: Barton LL, Abadia J (eds) Iron nutrition in plants and rhizospheric microorganisms. Springer, Dordrecht, pp 169–198

De Freitas JR, Banerjee MR, Germida JJ (1997) Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napus L.). Biol Fertil Soils 24:358–364

Dighton J, Boddy L (1989) Role of fungi in nitrogen, phosphorus and sulfur cycling in temperate forest ecosystems. In: Boddy L, Marchant R, Read D

(eds) Nitrogen, phosphorus and sulfur utilization by fungi. Cambridge University Press, Cambridge, pp 269–298

Di-Simine CD, Sayer JA, Gadd GM (1998) Solubilization of zinc phosphate by a strain of Pseudomonas fluorescens isolated from a forest soil. Biol Fertil Soils 28:87–94

Duponnois R, Kisa M, Plenchette C (2006) Phosphate solubilizing potential of the nematofungus *Arthrobotrys oligospora*. J Plant Nutr Soil Sci 169:280–282

Eivazi F, Tabatabai MA (1977) Phosphatases in soils. Soil Biol Biochem 9:167–172

Fabre B, Armau E, Etienne G, Legendre F, Tiraby G (1988) A simple screening method for insecticidal substances from actinomycetes. J Antibiot 41:212–219

Fankem H, Nwaga D, Deube A, Dieng L, Merbach W, Etoa FX (2006) Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. Afr J Biotechnol 5:2450–2460

Fenice M, Seblman L, Federici F, Vassilev N (2000) Application of encapsulated Penicilliumvariabile P16 in solubilization of rock phosphate. Bioresour Technol 73:157–162

Fraga R, Rodriguez H, Gonzalez T (2001) Transfer of the gene encoding the Nap A acid phosphatase from *Morganella morganii* to a *Burkholderia cepacia* strain. Acta Biotechnol 21:359–369

Gaur AC, Ostwal KP (1972) Influence of phosphate dissolving Bacilli on yield and phosphate uptake of wheat crop. Indian J Exp Biol 10:393–394

Gerretson FC (1948) The influence of microorganisms on the phosphorus uptake by plant. Plant Soil 1:51–81

Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–117

Goldstein AH (1994) Involvement of the quinoprotein glucose dehydrohenase in the solubilization of exogenous phosphates by Gram-negative bacteria. In: Torriani-Gorini A, Yagiland E, Silver S (eds) Phosphate in microorganisms: Cellular and molecular biology. ASM Press, Washington (DC), pp 197–203

Goldstein AH (1995) Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. Biol Agric Hortic 12:185–193

Goldstein AH, Liu ST (1987) Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. Biotechnology 5:72–74

Grierson PF, Comerford NB, Jokela EJ (1998) Phosphorus mineralization kinetics and response of microbial phosphorus to drying and rewetting in a Florida Spodosol. Soil Biol Biochem 30:1323–1331

Gunes A, Ataoglu N, Turan M, Esitken A, Ketterings QM (2009) Effects of phosphate-solubilizing microorganisms on strawberry yield and nutrient concentrations. J Plant Nutr Soil Sci 172:385–392

Gupta RR, Singal R, Shanker A, Kuhad RC, Saxena RK (1994) A modified plate assay for secreening phosphate solubilizing microorganisms. J Gen Appl Microbiol 40:255–260

Gyaneshwar P, Naresh KG, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83–93

Hamdali H, Bouizgarne B, Hafidi M, Lebrihi A, Virolle MJ, Ouhdouch Y (2008a) Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. Appl Soil Ecol 38:12–19

Hamdali H, Hafidi M, Virolle MJ, Ouhdouch Y (2008b) Growth promotion and protection against damping-off of wheat by two rock phosphate solubilizing actinomycetes in a P-deficient soil under greenhouse conditions. Appl Soil Ecol 40:510–517

Havlin J, Beaton J, Tisdale SL, Nelson W (1999) Soil fertility and fertilizers. An introduction to nutrient management. Prentice Hall, Upper Saddle River, NJ

He ZL, Wu J, O'Donnell AG, Syers JK (1997) Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soils under pasture. Biol Fertil Soils 24:421–428

Hwangbo H, Park RD, Kim YW, Rim YS, Park KH, Kim TH, Such JS, Kim KY (2003) 2-ketogluconic acid production and phosphate solubilization by Enterobacterintermedium. Curr Microbiol 47:87–92

Illmer PA, Schinner F (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soil. Soil Biol Biochem 24:389–395

Illmer PA, Schinner F (1995) Solubilization of inorganic calcium phosphates solubilization mechanisms. Soil Biol Biochem 27:257–263

Illmer PA, Barbato A, Schinner F (1995) Solubilization of hardly soluble AlPO4 with P-solubilizing microorganisms. Soil Biol Biochem 27:260–270

Isherwood KF (2000) Mineral Fertilizer Use and the Environment. International Fertilizer Industry Association/United Nations Environment Programme, Paris

Jacobs H, Boswell GP, Ritz K, Davidson FA, Gadd GM (2002) Solubilization of calcium phosphate as a consequence of carbon translocation by *Rhizoctonia solani*. FEMS Microbiol Ecol 40:65–71

Jorquera MA, Hernandez MT, Rengel Z, Marschner P, Mora MD (2008) Isolation of culturable phosphor bacteria with both phytate-mineralization and

phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. Biol Fertil Soils 44:1025–1034

Jorquera MA, Crowley DE, Marschner P, Greiner R, Ferna'ndez MT, Romero D, Menezes-Blackburn D, De La Luz Mora M (2011) Identification of b-propeller phytase-encoding genes in culturable Paenibacillus and bacillus sp. from the rhizosphere of pasture plants on volcanic soils. FEMS Microbiol Ecol 75:163–172

Juma NG, Tabatabai MA (1977) Effects of trace-elements on phosphatase-activity in soils. Soil Sci Soc Am J 41:343–346

Juma NG, Tabatabai MA (1998) Hydrolysis of organic phosphates by corn and soybean roots. Plant Soil 107:31–38

Khan MR, Khan SM (2002) Effect of root-dip treatment with certain phosphate solubilizing microorganisms. Bioresour Technol 85(2):213–215

Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed M (2009a) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J Agric Biol Sci 1(1):48–58

Khan MS, Zaidi A, Wani PA (2009b) Role of phosphate solubilising microorganisms in sustainable agriculture. In: Lictfouse E et al (eds) Sustainable Agriculture. springer, p 552, DOI: 10.1007/978-90-481-2666-8_34

Khan MS, Zaidi A, Wani PA, Oves M (2009c) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ chem lett 7:1–19

Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA (2010) Plant growth promotion by phosphate solubilizing fungi – current perspective. Arch Agron Soil Sci 56:73–98

Kim KY, McDonald GA, Jordan D (1997) Solubilization of hydroxyapatite by *Enterobacter agglomerans* and cloned Escherichia coli in culture medium. Biol Fertil Soils 24:347–352

Kim KY, Jordan D, McDonald GA (1998) *Enterobacter agglomerans*, phosphate solubilizing bacteria, and microbial activity in soil: effect of carbon sources. Soil Biol Biochem 30:995–1003

Krishnaraj PU, Khanuja SPS, Sadashivam KV (1998) Mineral phosphate solubilization (MPS) and mps genes -components in eco-friendly P fertilization. Abstracts of Indo US Workshop on Application of Biotechnology for Clean Environment and Energy, National Institute of Advanced Studies, Bangalore, p 27

Kucey RMN (1983) Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can J Soil Sci 63:671–678

Kumar V, Behl RK, Narula N (2001) Establishment of phosphate- solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. Microbiol Res 156:87–93

Lindsay WL, Vlek PLG, Chien SH (1989) Phosphate minerals. In: Dixon JB, Weed SB (eds) Minerals in soil environment, 2nd edn. Soil Science Society of America, Madison, WI, USA. pp, pp 1089–1130

Lopez BR, Bashan Y, Bacilio M (2011) Endophytic bacteria of *Mammillaria fraileana*, an endemic rock-colonizing cactus of the Southern Sonoran Desert. Arch Microbiol 193:527–541

Maliha R, Samina K, Najma A, Sadia A, Farooq L (2004) Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms under in vitro conditions. Pak J Biol Sci 7:187–196

McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26:267–268

Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In: Bunemann E, Oberson A, Frossard E (eds) Phosphorus in action: Biological processes in soil phosphorus cycling. Soil biology, 26. Springer, Heidelberg, pp 251–244

Nautiyal CS (1999) An efficient microbiological growth medium for screening of phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270

Norrish K, Rosser H (1983) Mineral phosphate. In: Soils: an Australian viewpoint. Academic Press, Melbourne, CSIRO/London, UK, Australia, pp 335–361

Oliveira CA, Sa NMH, Gomes EA, Marriel IE, Scotti MR, Guimaraes CT, Schaffert RE, Alves VMC (2009) Assessment of the mycorrhizal community in the rhizosphere of maize (Zea mays L.) genotypes contrasting for phosphorus efficiency in the acid savannas of Brazil using denaturing gradient gel electrophoresis (DGGE). Appl Soil Ecol 41:249–258

Omar SA (1998) The role of rock phosphate solubilizing fungi and vesicular arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J Microbiol Biotechnol 14:211–219

Park KH, Lee CY, Son HJ (2009) Mechanism of insoluble phosphate solubilization by Pseudomonas fluorescens RAF15 isolated from ginseng rhizosphere and its plant growth-promoting activities. Lett Appl Microbiol 49:222–228

Parker DR, Reichmann SM, Crowley DE (2005) Metal chelation in the rhizosphere. In: Zobel RW (ed) Roots and soil management: interactions between roots and the soil. Agronomy monograph no. 48. American Society of Agronomy, Madison, pp 57–93

Parks EJ, Olson GJ, Brinckman FE, Baldi F (1990) Characterization by high performance liquid chromatography (HPLC) of the solubilization of phosphorus in iron ore by a fungus. J Ind Microbiol Biotechnol 5:183–189

Patil MG, Sayyed RZ, Chaudhari AB, Chincholkar SB (2002) Phosphate Solubilizing Microbes: A Potential Bioinoculant for Efficient Use of Phosphate Fertilizers. In: Reddy SM, Reddy SR, Grisham S (eds) Bioinoculants for Sustainable Agriculture and Forestry. Scientific Publisher, Jodhpur, pp 107–118

Peix A, Velazquez E, Martýnez-Molina E (2007) Molecular methods for biodiversity analysis of phosphate solubilizing microorganisms (PSM). In: Velazquez E, Rodrguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Springer, Berlin, pp 97–100

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiology 17:362–370

Puente ME, Bashan Y, Li CY, Lebsky VK (2004a) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. Plant Biol 6:629–642

Puente ME, Li CY, Bashan Y (2004b) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. Plant Biol 6:643–650

Puente ME, Li CY, Bashan Y (2009a) Rock-degrading endophytic bacteria in cacti. Environ Exp Bot 66:389–401

Puente ME, Li CY, Bashan Y (2009b) Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. Environ Exp Bot 66:402–408

Quiquampoix H, Mousain D (2005) Enzymatic hydrolysis of organic phosphorus. In: Turner BL, Frossardand E, Baldwin DS (eds) Organic phosphorus in the environment. CAB International, Wallingford UK, pp 89–112

Renella G, Egamberdiyeva D, Landi L, Mench M, Nannipieri P (2006) Microbial activity and hydrolase activities during decomposition of root exudates released by an artificial root surface in Cd-contaminated soils. Soil Biol Biochem 38:702–708

Rengel Z, Marschner P (2005) Nutrient availability and management in the rhizosphere: exploiting genotypic differences. New Phytology 168:305–312

Reyes I, Bernier L, Simard RR, Antoun H (1999) Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV-induced mutants. FEMS Microbiol Ecol 28:281–290

Reyes I, Baziramakenga R, Bernier L, Antoun H (2001) Solubilization of phosphate rocks and minerals by a wild type strain and two UV induced mutants of *Penicillium regulosum*. Soil Biol Biochem 33:1741–1747

Reyes I, Bernier L, Antoun H (2002) Rock phosphate solubilization and colonization of maize rizosphere by wild and genetically modified strains of *Penicillium rugulosum*. Microb Ecol 44:39–48

Richardson AE (1994) Soil microorganisms and phosphorus availability. In: Pankhurst CE, Doubeand BM, Gupta VVSR (eds) Soil biota: management in sustainable farming systems. CSIRO, Victoria, Australia, pp 50–62

Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust J Plant Physiol 28:897–906

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability. Plant Physiol 156:989–996

Richardson AE, Hadobas PA, Hayes JE, O'Hara CP, Simpson RJ (2001) Utilization of phosphorus by pasture plants supplied with myo-inositol hexaphosphate is enhanced by the presence of soil microorganisms. Plant Soil 229:47–56

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009a) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305–339

Richardson AE, Hocking PJ, Simpson RJ, George TS (2009b) Plant mechanisms to optimize access to soil phosphorus. Crop Pasture Sci 60:124–143

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21

Rudolph W (1922) Influence of S oxidation upon growth of soybeans and its effect on bacterial flora of soil. Soil Sci l4:247–263

Saber K, Nahla LD, Chedly A (2005) Effect of P on nodule formation and N fixation in bean. Agron Sustain Dev 25:389–393

Sanchez P, Logan T (1992) Myths and science about the chemistry and fertility of soils in the tropics. In: Lal R, Sanchez P (eds) Myths and science of soils of the tropics. Soil Science Society of America, Madison, WI, pp 35–46

Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson MJ, Beaty KG, Lyng M, Kasian SEM (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proc Natl Acad Sci U S A 105:11254–11258

Sims JT, Pierzynski GM (2005) Chemistry of phosphorus in soil. In: Tabatabai AM, Sparks DL (eds) Chemical processes in soil, SSSA book series 8. SSSA, Madison, pp 151–192

Singal R, Gupta R, Saxena RK (1994) Rock phosphate solubilization under alkaline conditions by *Aspergillus japonicus* and *A. foetidus*. Folia Microbiol 39:33–36

Song OR, Lee SJ, Lee YS, Lee SC, Kim KK, Choi YL (2008) Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* Da23 isolated from cultivated soil. Braz J Microbiol 39:151–156

Sperber JI (1958a) The incidence of apatite-solubilizing organisms in the rhizosphere and soil. Aust J Agr Res 9:778–781

Sperber JI (1958b) Solubilization of apatite by soil microorganisms producing organic acids. Aust J Agr Res 9:782–787

Sridevi M, Mallaiah KV, Yadav NCS (2007) Phosphate solubilization by *Rhizobium* isolates from Crotalaria species. J Plant Sci 2:635–639

SubbaRao NS (1982) Advances in agricultural microbiology. Oxford and IBH Publications Company, India, pp 229–305

Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147:3–9

Swaby R, Sperber JI (1958) Phosphate Dissolving Microorganisms in the Rhizosphere of Legume, Nutrition Of Legumes; Proc. Univ. Nottingham 5Th Easter Sch. Agril. Sci. (CSIRO Adelaide). Soils & Fert 22, 286(1959):289–294

Taha SM, Mahmoud SAZ, El-Damaty AA, Abd El- Hafez AM (1969) Activity of phosphate dissolving bacteria in Egyptian soil. Plant Soil 31:149

Tarafdar JC, Yadav RS, Meena SC (2001) Comparative efficiency of acid phosphatase originated from plant and fungal sources. J Plant Nutr Soil Sci 164:279–282

Tilman D, Fargione J, Wolff B, D'Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Simberloff D, Wackhamer D (2001) Forecasting agriculturally driven global environmental change. Science 292:281–284

Torsvik V, Ovreas L (2002) Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5:240–245

Trolove SN, Hedley MJ, Kirk GJD, Bolan NS, Loganathan P (2003) Progress in selected areas of rhizosphere research on P acquisition. Aust J Soil Res 41:471–499

Vassilev N, Vassileva M, Azcon R, Medina A (2001) Preparation of gel-entrapped mycorrhizal inoculum in the presence or absence of *Yarrowia lypolytica*. Biotechnol Lett 23:907–909

Vassilev N, Vassileva M, Nikolaeva I (2006) Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Appl Microbiol Biotechnol 71:137–144

Vazquez P, Holguin G, Puente M, Lopez-cortes A, Bashan Y (2000) Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semi-arid coastal lagoon. Biol Fertil Soils 30:460–468

Venkateswarlu B, Rao AV, Raina P, Ahmad N (1984) Evaluation of phosphorus solubilization by microorganisms isolated from arid soil. J Indian Soc Soil Sci 32:273–277

Vyas P, Gulati A (2009) Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. BMC Microbiol 9:174

Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004) Phosphate solubilization by *Penicillium* sp. closely associated with wheat roots. Biol Fertil Soils 40:36–43

Wani PA, Zaidi A, Khan AA, Khan MS (2005) Effect of phorate on phosphate solubilization and indole acetic acid (IAA) releasing potentials of rhizospheric microorganisms. Annals Plant Protection Sci 13:139–144

Whitelaw MA (2000) Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv Agron 69:99–151

Whitelaw MA, Harden TJ, Helyar KR (1999) Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum*. Soil Biol Biochem 32:655–665

Widada J, Damarjaya DI, Kabirun S (2007) The interactive effects of arbuscular mycorrhizal fungi and rhizobacteria on the growth and nutrients uptake of sorghum in acid soil. In: Rodriguez-Barrueco C (ed) Velazquez E. First International Meeting on Microbial Phosphate Solubilization, Springer, pp 173–177

Yi Y, Huang W, Ge Y (2008) Exopolysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. World J Microbiol Biotechnol 24:1059–1065

Zaidi A, Khan MS, Ahemad M, Oves M, Wani PA (2009) Recent Advances in Plant Growth Promotion by Phosphate-Solubilizing Microbes. In: Khan MS et al (eds) Microbial Strategies for Crop Improvement. Springer-Verlag, Berlin Heidelberg, pp 23–50

Zhou K, Binkley D, Doxtader KG (1992) A new method for estimating gross phosphorus mineralization and immobilization rates in soils. Plant Soil 147:243–250

Zhu F, Qu L, Hong X, Sun X (2011) Isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the coast of Yellow Sea of China. Evid base Compl Alternative Med 615032:6