Chapter 11
Phosphorus Cycling: Prospects of Using Rhizosphere Microorganisms for Improving Phosphorus Nutrition of Plants

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11.1 Introduction

Phosphorus (P) is a major essential macronutrient for biological growth and development. It is an essential element found in all living beings as part of proteins, nucleic acids, membranes, and energy molecules such as ATP, GTP, and NADPH. It is involved in many cellular essential processes including cell division, photosynthesis, breakdown of sugar, energy transfer, nutrient transport within the plant, expression and maintenance of genetic material, and regulation of metabolic pathways. In agriculture, P is the second major nutrient element in terms of quantitative requirement limiting plant growth preceded by nitrogen (Hinsinger 2001; Fernandez et al. 2007). It is found in soil, plants, and microorganisms in a number of organic and inorganic compounds. However, the total P content in an average soil is 0.05 % and only 0.1 % of the total P present in the soil is available to the plants. Even though some soils may have high levels of total P, they can still be P deficient due to low levels of soluble phosphate available to plants (Gyaneshwar et al. 2002). Thus, the pool of immediately available P must be replenished regularly to meet plant requirements (Bieleski 1973; Richardson and Simpson 2011).

Phosphorus deficiency in soil is traditionally overcome by adding either phosphatic fertilizers (Khan et al. 2006) or it may be incorporated as leaf litter, plant residues, or animal remains. The phosphatic fertilizers are the world's second largest bulk chemical used in agriculture on earth (Goldstein et al. 1993; Goldstein 2007). After the addition of chemical phosphatic fertilizers, the extremely reactive soluble phosphate anions ($H_2PO_4^-$, HPO_4^{2-}) may form metal complexes with Ca in calcareous soils (Lindsay et al. 1989) and Fe^{3+} and Al^{3+} in acidic soils (Norrish and Rosser 1983). Thus, a large portion, i.e., 75–90 % of added P fertilizer in

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agricultural soils is precipitated/immobilized rapidly by iron, aluminum, manganese, and calcium complexes depending on soil type, soil pH, and existing minerals (Richardson et al. 2001b; Bünemann et al. 2006; Vu et al. 2008). Generally, a few days after fertilization, available phosphate levels can reach similar values to those before application (Sharpley 1985). Thus, due to the low P fertilizer efficiency, farmers often apply P fertilizers in excess of plant requirement to sustain crop production (Rodriguez and Fraga 1999) and this practice has resulted in a buildup of residual P and nonlabile inorganic P in the soil (Vu et al. 2008) leading to environmental problems such as water eutrophication (Correll 1998) and soil pollution. In order to meet current demands of food production by improving crop productivity, enhanced fertilization has provoked an intense scavenging of phosphorus mines worldwide and it is estimated that by 2060 these mines could be depleted (Gilvert 2009; Cordell et al. 2009). Therefore, there is an urgent need to explore alternative sources for better management of plant–soil–microbial P cycle to reduce our reliance on mineral fertilizers.

Utilization of microorganisms is an attractive approach to increase the availability of P in soil leading to enhanced crop production and to develop a more sustainable agricultural system under recent intensive, nutrient-extracting agricultural practices (Sanchez et al. 1997; Deubel and Merbach 2005; Richardson and Simpson 2011). The use of phosphate-solubilizing microorganisms (PSMs) is economical, ecofriendly, and has greater agronomic utility to compensate the expensive inorganic sources of P fertilizers. Thus, association between plant roots and phosphate-solubilizing microorganisms could play an important role in P nutrition in many natural agroecosystems (Rodriguez and Fraga 1999; Bagyaraj et al. 2000; Richardson et al. 2001b). Many phosphate-solubilizing bacteria including *Alcaligenes*, *Arthrobacter*, *Azotobacter*, *Bradyrhizobium*, *Bacillus*, *Burkholderia*, *Chromobacterium*, *Enterobacter*, *Erwinia*, *Escherichia*, *Flavobacterium*, *Micrococcus*, *Pantoea*, *Pseudomonas*, *Salmonella*, *Serratia*, *Streptomyces*, and *Thiobacillus* have been isolated (Zhao and Lin 2001; Sindhu et al. 2009; Castagno et al. 2011; Azziz et al. 2012). Efficient phosphate-solubilizing fungi include the genus *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, and *Sclerotium* (Zhao and Lin 2001). Inoculation of many phosphate-solubilizing microorganisms has been found to support growth of plants under nutrient imbalance conditions (Glick 1995; Igual et al. 2001; Wu et al. 2005).

Microbial-mediated solubilization of insoluble phosphates in the cultivated soils is generally attributed to production of organic acids by microorganisms (Kim et al. 1998a; Carrillo et al. 2002; Rodriguez et al. 2004). Organic acids including acetate, lactate, malate, oxalate, succinate, citrate, gluconate, ketogluconate, etc., can form complexes with the iron or aluminum in ferric and aluminum phosphates, thus releasing plant available phosphate into the soil (Jones 1998; Gyaneshwar et al. 2002). Moreover, the release of organic acid anions such as malate and citrate can mobilize soil P pools by reducing the number of binding sites for P fixation via chelation of Fe and Al (Gerke 1992) and by replacing P from adsorption sites (Nziguheba et al. 2000). Besides, the release of enzymes such as acid phosphatase

(Tarafder and Claassen 2003) and phytase (Richardson 2001) plays an important role in mobilization of organic P.

Recently, modest application of rock phosphate (RP) along with inoculation of phosphate-solubilizing microorganisms has been found to enhance P availability in soils with very low P status (Jones and Oburger 2011; Arcand and Schneider 2006). Thus, solubilization of RP by soil microorganisms serves a source of phosphorus for crops at lower cost with less technological sophistication. Therefore, an enormous amount of research has been conducted recently on isolation and characterization of PSMs from different soils with the objective of developing phosphatic biofertilizers. Considering the fact that world's high-quality sources of rock phosphate are finite and are distributed over only to few countries such as Morocco, the USA, China, Russia, etc., the world's supply of fossil resources is shrinking with the increasing demand of phosphatic fertilizers. This justifies the need to develop plants and/or agricultural systems that are more P efficient. For example, plant species particularly legumes, are capable of mobilizing P from less labile P pools than cereals (Kamh et al. 1999; Nuruzzaman et al. 2005a, b). Similarly, efficient phosphate solubilizing microorganisms could be used as inoculant for improving plant growth of agriculture and horticulture crops (Bagyaraj et al. 2000; Gyaneshwar et al. 2002; Khan et al. 2007; Naik et al. 2008). This chapter aims to identify the phosphate-solubilizing microorganisms from soil or rhizosphere and to understand the mechanism used for P solubilization. The inoculation responses of the phosphate-solubilizing microorganisms and their genetic manipulations to improve P solubilization capacity are presented in this chapter to improve the quality of agricultural inoculants for achieving enhanced crop productivity.

11.2 Phosphorus Cycling and Availability in Soils

The important reservoir of immobilized P in the soil is organic matter (Richardson 1994). The organic compounds making up the humus fraction are derived from surface vegetation, microbial protoplasm, or metabolic products of the microflora. The various inositol phosphates are often classified together as phytin or related substances and such organic matter components frequently accounts for 20–80 % of the entire organic P fraction. The phospholipid content of humus is invariably small and often 0.1–5 % or sometimes slightly more of the organic phosphorus is tied up in such compounds. A significant part of phospholipids may be phosphatidyl ethanolamine and phosphatidyl choline and these compounds are found in both plants and microorganisms. Phosphorus held within soil microorganisms constitutes a significant component of the total soil P and is estimated to account for around 2–10 % of total soil P. However, at different stages of soil development and within litter layers (soil surface), this may be as much as 50 % (Oberson and Joner 2005; Achat et al. 2010). Usually, soils rich in organic matter contain abundant organic P. Moreover, a good correlation exists between the concentrations of organic P, organic C, and total N. Ratios of organic C to organic P of 100–300:1

are common for mineral soils. Similarly, the nitrogen: organic phosphorus ratio may range from 5 to 20 parts of nitrogen for each part of P. The organic P level, therefore, is directly related to the concentration of other humus constituents, the P content being 0.3–1.0 % and 5–20 % of the C and N concentration, respectively.

Besides organic P, large quantities of the inorganic forms of P occur in minerals where the phosphate is part of the mineral structure, as insoluble calcium, iron or aluminum phosphates (Richardson et al. 2001b; Turan et al. 2006; Vu et al. 2008). Under acidic conditions, P ions are present as H_2PO_4 but are subjected to fixation with hydroxides of Al and Fe at pH below 5. Near neutral pH, HPO_4^{2-} ions are usually present. But above pH 8, the PO_4^{3-} ions form $[Ca_3(PO_4)_2]$ and its availability is reduced drastically. The P nutrient is estimated to be in insufficient amounts in most of the Indian soils as available P. According to the compilation of about 9.6 million soil tests for available P in Indian soils, it was reported that 49.3 % of areas covering different states and union territories are in the low category, 48.8 % in the medium category, and 1.9 % have high phosphorus status (Hasan 1994). Therefore, application of phosphatic fertilizers is unavoidable in intensive farming system. The source of P is only from phosphatic and sulfur rocks, which are nonrenewable sources and use of phosphatic fertilizers leads to the depletion of these resources. Thus, problem of P management in soil is also very tricky and more than 70–90 % of the applied phosphatic fertilizers get fixed in the soil rendering them unavailable for plant uptake under the ideal conditions (Larsen 1967; Holford 1997).

The role of the microbial biomass in the cycling of P in soil has recently received increased attention (Oberson and Joner 2005). Soil microorganisms effectively compete with plants for available orthophosphate from soil solution and also represent a significant pool of immobilized P that is temporarily unavailable to plants. However, significant amounts of P can be released from the microbial biomass in response to seasonal conditions when either carbon becomes limiting or soils undergo cycles of wetting and drying (Turner and Haygarth 2001; Bonkowski 2004). To be available to plants, orthophosphate must diffuse through the rhizosphere (Jakobsen et al. 2005) and as such will be in direct competition for uptake and immobilization by microorganisms. Subsequently, the rate of release of P from microorganisms or the turnover time for the microbial biomass within the rhizosphere will have major implication for P availability to plants. Radioactive-tracer studies indicated that orthophosphate released through microbial turnover contributes significantly to basal rates of mineralization in soil and estimations suggest a turnover time of the total microbial biomass in bulk soil of between 42 and 160 days depending on the farming system, whereas faster rates of turnover were observed in C-amended soils (Oehl et al. 2004; Bünemann et al. 2007). Achat et al. (2010) reported a faster cycling of a major component of the soil microbial P pool (accounting for 80 % of the total microbial P), with a turnover time of less than 10 days in an organic P-dominated forest soil. Recently, Bünemann et al. (2012) measured gross phosphorus fluxes in isotopic dilution studies with ^{33}P-labeled soils that included the biological processes of microbial P immobilization, remineralization of immobilized P, and mineralization of nonmicrobial soil organic P. The

results showed that inorganic P availability primarily affected microbial P immobilization which was the main component of gross P fluxes in both treatments.

Legumes have the capacity to mobilize more P from less residual inorganic P than cereals (Nuruzzaman et al. 2005b; Vu et al. 2008) and different legumes also differed in their capacity to utilize residual inorganic P from the rhizosphere. Hassan et al. (2012) compared the growth, P uptake, and the changes in rhizosphere soil P pools in five grain legumes in a soil with added P. Nodulated chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), white lupin (*Lupinus albus* L.), yellow lupin (*Lupinus luteus* L.), and narrow-leafed lupin (*Lupinus angustifolius* L.) were grown in a loamy sand soil low in available P to which 80 mg P kg^{-1} was added and harvested at flowering and maturity. At maturity, growth and P uptake decreased in the following order: faba bean > chickpea > narrow-leafed lupin > yellow lupin > white lupin. Compared to the unplanted soil, the depletion of labile P pools (resin P and $NaHCO_3$-P inorganic) was greatest in the rhizosphere of faba bean (54 % and 39 %). Of the less labile P pools, NaOH-P inorganic was depleted in the rhizosphere of faba bean, while NaOH-P organic and residual P was most strongly depleted in the rhizosphere of white lupin. The results suggested that even in the presence of labile P, less labile P pools may be depleted in the rhizosphere of some legumes.

11.3 Microorganisms Involved in Solubilization of Inorganic Phosphorus

The insoluble phosphates predominant in saline and saline alkaline soils include tricalcium phosphate [$Ca_3(PO_4)_2$], carbonate apatites [$Ca_3(PO_4)_2{\cdot}CaCO_3$], hydroxy apatites [$Ca_3(PO_4)_2{\cdot}Ca(OH)_2$], oxi apatites [$Ca(PO_4)_2{\cdot}CaO$], and fluor apatites [$Ca_3(PO_4)_2{\cdot}CaF_2$], whereas hydroxyl phosphates of Fe and Al namely dufrenite, strengite [$Fe(OH)_2H_2PO_4$], varisite [$Al(OH)_2H_2PO_4$], etc., are usually present in acidic soils. These unavailable forms are converted to primary orthophosphate ($H_2PO_4^-$) and secondary orthophosphates ($H_2PO_4^{-2}$), which are available for plant growth. The ability of soil or rhizosphere bacteria to solubilize mineral phosphates is generally screened on a solid medium containing insoluble phosphate source such as tricalcium phosphate (TCP), apatite, rock phosphate (RP) and, in some cases, Fe and Al phosphates in agar media. The appearance of clearing zones around colonical growth of microorganisms indicates the ability to release Pi from the precipitate of insoluble phosphate and these bacterial strains are considered positive for P solubilization activity. Indicator medium containing dyes such as bromothymol blue (Krishanaraj 1996) or bromocresol green could also be used for better observation (Mehta and Nautiyal 2001; Gadagi and Tongmin 2002). The solubilization of different types of insoluble phosphates varies with the type of microorganisms, the type of phosphates available, media conditions, and available carbon source.

Stalstorm in 1903, first time demonstrated solubilization of TCP by soil bacteria in liquid and on solid media. Since then, a large number of heterotrophic and autotrophic soil microbes representing bacterial, actinomycetes, and fungal species have been identified as active P solubilizers. About 10–50 % of the bacterial isolates tested are capable of solubilizing calcium phosphates and counts of bacteria solubilizing insoluble P may range from 10^5 to 10^7 per gram of soil. Kucey et al. (1989) reported that PSM were present in almost all the soils although their number varied depending upon the soil and climatic conditions. PSM have been isolated from different sources such as, soil (Roychaudhary and Kaushik 1989), rhizosphere (Thakkar et al. 1993), compost (Thakkar et al. 1993; Gupta et al. 1993), rock phosphate (Bardiya and Gaur 1972; Gaur et al. 1973), and root nodules (Halder et al. 1991; Surange and Kumar 1993). The bacteria characterized as active phosphate solubilizers represented diverse groups ranging from autotrophs to heterotrophs, diazotrophs to phototrophs; fungi including mycorrhizal fungi both ectotrophic as well as endotrophic, and actinomycetes. Higher populations of bacteria and fungi capable of dissolving insoluble P were observed in the rhizosphere and rhizoplane of different crops as compared to non-rhizosphere soil (Katznelson and Bose 1959; Puente et al. 2004; Fankem et al. 2006). Tomar (2005) reported that the counts of phosphate-solubilizing bacteria (PSB) were more in chickpea rhizosphere followed by wheat and mustard. These PSB isolates showed large variation in P solubilization on Pikovskaya's medium.

The most important phosphate-solubilizing bacteria belong to genera *Bacillus* and *Pseudomonas*, though species of *Achromobacter*, *Alkaligenes*, *Brevibacterium*, *Corynebacterium*, *Serratia*, and *Xanthomonas* have also been found active in solubilizing insoluble P (Venkateswarlu et al. 1984). Phosphate-solubilizing *Pseudomonas* species isolated from rhizosphere of leguminous and cereal crops include *P. aeruginosa*, *P. chlororaphis*, *P. fluorescens*, *P. liquifaciens*, *P. pickettii*, *P. putida*, *P. rathonis*, *P. savastanoi*, *P. striata*, and *P. stutzeri* (Rajarathinam et al. 1995; Cattelan et al. 1999). Naik et al. (2008) screened 443 fluorescent pseudomonad strains for the solubilization of tricalcium phosphate and reported that 80 strains (18 %) formed visible dissolution halos on Pikovskaya agar medium plates. Based on phenotypic characterization and 16S rRNA gene phylogenetic analyses, strains were identified as *Pseudomonas aeruginosa*, *P. mosselii*, *P. monteilii*, *P. plecoglosscida*, *P. putida*, *P. fulva* and *P. fluorescens*. The phosphate-solubilizing *Bacillus* species isolated from the rhizosphere of legumes and cereals like rice, maize, and oat, jute, and chilli include *Bacillus subtilis*, *B. circulans*, *B. coagulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, and *B. polymyxa* (Barea et al. 1976; Gaind and Gaur 1991; Rajarathinam et al. 1995). Other P-solubilizing bacteria include species of bacteria like *Acinetobacter*, *Azotobacter chroococcum*, *Burkholderia cepacia*, *Erwinia herbicola*, *Enterobacter agglomerans*, *E. aerogenes*, *Kushneria* sp., *Nitrosomonas*, *Nitrobacter*, *Serratia marcescens*, *Synechococcus* sp., *Rahnella aquatilis*, *Micrococcus*, *Thiobacillus ferroxidans*, and *T. thiooxidans* (Banik and Dey 1983c; Kim et al. 1998a; Sheshardri et al. 2000; Zhu et al. 2011; Azziz et al. 2012). *Rhizobium* and *Bradyrhizobium* strains have also been found to solubilize RP or organic P

compounds effectively through the production of organic acids and/or phosphatases (Halder et al. 1991; Abd-Alla 1994).

Castagno et al. (2011) obtained 50 isolates from Salado river basin and 17 nonredundant strains were identified through BOX-PCR analysis. They were found to be related to *Pantoea*, *Erwinia*, *Pseudomonas*, *Rhizobium*, and *Enterobacter* genera via 16S rRNA gene sequence analysis. Viruel et al. (2011) characterized phosphobacteria from Puna, northwestern Argentina, and P-solubilizing activity was found to coincide with a decrease in pH values of the tricalcium phosphate medium for all strains after 72 h of incubation. Identification by 16S rDNA sequencing and phylogenetic analysis revealed that these strains belong to the genera *Pantoea*, *Serratia*, *Enterobacter*, and *Pseudomonas*. A moderately halophilic phosphate-solubilizing bacterium *Kushneria* sp. YCWA18 was isolated from the sediment of Daqiao saltern on the eastern coast of China (Zhu et al. 2011). The fastest growth of PSB was observed when the culturing temperature was 28 °C and the concentration of NaCl was 6 % (w/v). It was found that the bacterium can survive at a concentration of NaCl up to 20 %. The bacterium solubilized 283.16 μg ml^{-1} phosphorus in 11 days after being inoculated in 200 ml $Ca_3(PO_4)_2$ containing liquid medium and 47.52 μg ml^{-1} phosphorus in 8 days after being inoculated in 200 ml lecithin-containing liquid medium. The growth of the bacterium was concomitant with a significant decrease of acidity of the medium. Prasanna et al. (2011) selected thirty efficient PSB isolates among 226 colonies showing clear zone formation on Pikovskaya's agar medium, which were isolated from rice rhizosphere soils of Southern peninsular region of India. The isolated PSB strains released high amount of phosphorus from tricalcium phosphate and it ranged from 22.4 to 825.8 μg P ml^{-1} and the amount of phosphatase secreted into the medium ranged from 11.6 to 64 U. The efficient strains isolated from various rhizosphere soils were identified as *Enterobacter*, *Micrococcus*, *Pseudomonas*, *Bacillus*, *Klebsiella*, and *Serratia*. Among all the strains, A4 strain (*Enterobacter aerogenes*) released high amount of phosphorus.

Chookietwattana and Maneewan (2012) screened 84 halotolerant bacterial strains for solubilization of insoluble phosphate in the modified Pikovskaya broth and *Bacillus megaterium* strain A12ag showed highest phosphate solubilization under saline conditions. Panhar et al. (2012) showed that PSB populations were higher in rhizosphere of aerobic rice than non-rhizospheric soil and the highest population was found in Pikovskaya and *Pseudomonas* spp. (PS) medium, while the lowest was found in *Pseudomonas aeruginosa* (PA) medium plates. The highest P-solubilizing activity (69.58 %) was found in PSB9 strain grown in national botanical research institute's phosphate growth medium (NBRIP) plate. Singh et al. (2012) screened 35 bacterial isolates for their phosphate-solubilizing ability and 2 of them were identified through 16S rDNA sequencing as *Chryseobacterium* sp. PSR10 and *Escherichia coli* RGR13, respectively. Azziz et al. (2012) examined the abundance and diversity of phosphate-solubilizing bacteria (PSB) in a crop/pasture rotation experiment in Uruguay. The percentage of PSB relative to total heterotrophic bacteria ranged between 0.18 and 13.13 % and 12 isolates showed greatest solubilization activity and were characterized by 16S rDNA sequencing,

10 isolates belonged to the genus *Pseudomonas*, and 2 isolates showed high similarity with members of the genera *Burkholderia* and *Acinetobacter*. Shahid et al. (2012) isolated an *Enterobacter* sp. Fs-11 from sunflower (GeneBank accession no. GQ179978), which converted insoluble tricalcium phosphate to soluble phosphorus up to 43.5 μg ml^{-1} with decrease in pH of the medium up to 4.5 after 10 days incubation at 28 ± 2 °C in the Pikovskaya's broth.

The important P-solubilizing fungi belonged to genus *Aspergillus* and *Penicillium* (Asea et al. 1988; Reyes et al. 1999; Rashid et al. 2004). A few species of *Fusarium oxysporum*, *Trichoderma viride*, *Curvularia lunata*, *Sclerotium rolfsii*, *Alternaria teneuis*, *Humicola*, *Pythium*, *Phoma*, *Acrothecium*, *Morteirella*, *Paecilomyces*, *Rhizoctonia*, *Rhodotorula*, *Candida* sp., *Cunninghamella*, *Oideodendron*, *Pseudogymnoascus*, and *Trichoderma viride* were also found as good solubilizers of insoluble P. *Torula* sp. which are usually not present in soil, have been isolated from compost, and have been characterized for solubilization of TCP and RP by Singh et al. (1980). Among actinomycetes, *Actinomyces*, *Micromonospora*, *Nocardia*, and *Streptomyces* have been reported to solubilize mineral phosphate (Banik and Dey 1983a).

Recently, Tallapragada and Seshachala (2012) studied the native populations of phosphate-solubilizing bacteria and fungi in different rhizospheric soil samples obtained from betel vine plants (*Piper betel* L.). The phosphate-solubilizing capacity of bacteria and fungi revealed the dominance of *Aspergillus* species (26.1 mm) as major phosphate solubilizers, along with *Bacillus subtilis* (46.6 mm) among the bacteria that utilize tricalcium phosphate, potassium dihydrogen phosphate, and rock phosphate as phosphate sources. The other phosphorus-solubilizing microorganisms were *Bacillus* species, *Streptomyces*, *Aspergillus fumigatus*, *Nocardia*, actinomycetes, and certain yeasts. The population of phosphate-solubilizing bacterium *Bacillus subtilis* was 3×10^6 cfu g^{-1} and the population of fungus *Aspergillus niger* was 3×10^5 cfu g^{-1} in the rhizospheric zones of *Piper betel* plants.

The comparative solubilization pattern observed by the use of different PSM showed that TCP is most easily solubilized followed by ferric, aluminum, and RP (Banik and Dey 1981; Gaind and Gaur 1990; Kole and Hazra 1998). Strains of *Pseudomonas* spp. are capable of releasing 160.5–162.5 μg ml^{-1} in the medium containing TCP (Santhi 1998). Strains of *Acetobacter diazotrophicus* isolated from sugarcane were found to release 142–431 μg ml^{-1} Pi from TCP (Maheshkumar et al. 1999). The solubilization of TCP in liquid medium by different fluorescent *Pseudomonas* strains varied from 29 to 105 μg ml^{-1} on 10 days of inoculation and a significant drop in pH of Pikovskaya liquid medium was observed on 10 days of inoculation (Naik et al. 2008). Estimations of phosphate solubilization of different bacterial strains by other methods have been reported to range between 200 and 805 μg ml^{-1} (Nautiyal 1990). *P. fluorescens* strain NJ-101 isolated from agricultural soil was reported to release 74.6 μg ml^{-1} soluble phosphate from inorganic phosphate (Bano and Musarrat 2004). *Enterobacter agglomerans* strains were found to release Pi ranging from 82.6 to 551.3 μg ml^{-1} in medium containing hydroxyapatite (Kim et al. 1997). *Pseudomonas striata* has been reported to be more efficient than *Bacillus* spp. and *Aspergillus awamorii* in solubilizing TCP.

P. putida solubilized TCP to the extent of 50 % (Ostwal and Bhide 1972). Varsha et al. (1994) found that *Aspergillus awamorii* was best in solubilizing TCP (94 %) followed by dicalcium phosphate (54.5 %) and aluminum phosphate (31.8 %). However, ferric phosphate was best solubilized by *Aspergillus niger*.

Many bacteria capable of dissolving tricalcium phosphate fail to solubilize RP (Bardiya and Gaur 1972) and the organic phosphate-mineralizing bacteria or fungi do not prove to be efficient solubilizer of RP (Gaur et al. 1973). Among the different types of RP tested, Gufsa rock phosphate was solubilized maximum followed by Morocco, Jordan, Udaipur, Singhbhum, and Mussoorie rock phosphate (Singh et al. 1984). The growth and population of phosphate solubilizers was correlated with the extent of phosphate solubilized. Similarly, among China, Senegal, Hirapur, Udaipur, and Sonrai rock phosphate, Senegal rock phosphate was most efficiently solubilized by *Rhodotorula minuta* and *Saccharomyces cerevisiae* (Varsha and Patel 1995). Therefore, for effective solubilization of different phosphate types found in soil, it will be worthwhile to isolate rock phosphate dissolving microorganisms by enrichment culture techniques from such soils.

11.4 Mechanisms of Phosphorus Solubilization by Soil Microorganisms

Organic acids and protons are particularly effective in solubilizing precipitated or complexed forms of soil P or by facilitating the release of adsorbed orthophosphate or organic P through ligand exchange reactions (Ryan et al. 2001). Such mechanisms are widely demonstrable under laboratory and, in some cases, under controlled glasshouse conditions. However, their operation and quantification in field soils to directly supply P to plants is more difficult to assess. Moreover, plants themselves display a wide array of root morphological and physiological changes in response to P deficiency (Vance et al. 2003; Richardson et al. 2009b) and thus assessment of microbial verses plant-mediated processes for P mobilization is difficult. Nonetheless, microorganisms are integral to the cycling of soil P and enhancement of microbial activity in the rhizosphere has significant implication for the P nutrition of plants.

11.4.1 Solubilization of Inorganic Phosphorus

The amount of P solubilized under cultural conditions is dependent on the composition of the media and form of inorganic P precipitate used (including Ca-, Fe-, and Al-phosphates and various sources of rock phosphate) along with cultural and sampling procedures. Different mechanisms are employed by various phosphate solubilizing bacterial strains to solubilize bound form of phosphorus.

11.4.1.1 Production of Organic Acids

In most bacteria, mineral phosphate-solubilizing capacity has been shown to be related to the production of organic acids (Rodriguez and Fraga 1999; Shahid et al. 2012). Analyses of supernatants of growth of many phosphate-solubilizing bacteria showed the production of mono-, di-, and tricarboxylic acids (Table 11.1). The amount of acids liberated by these bacteria is more than 5 % of the carbohydrate consumed (Banik and Dey 1983a). A direct correlation between drop in pH and increase in available P of the culture media has been observed in certain cases (Agnihotri 1970; Liu et al. 1992). The most commonly produced acids include citric, fumaric, lactic malic, glyoxalic, succinic, tartaric, and α-ketobutyric acid secreted by *Bacillus megaterium*, *B. circulans*, *E. freundii*, *and Pseudomonas striata*. High performance liquid chromatography of cell-free supernatant of phosphate-solubilizing bacterium *Enterobacter* sp. Fs-11 showed that it produced malic acid and gluconic acid (2.43 and 16.64 μg ml^{-1}, respectively) in Pikovskaya's broth (Shahid et al. 2012). However, the fungi *A. awamorii* and *P. digitatum* were found to synthesize citric, succinic, and tartaric acid (Banik and Dey 1983a).

Glucose-derived gluconic acid (GA) produced in the periplasmic space of Gram-negative bacteria resulted in decrease of pH and seems to directly correlate with the phosphate-solubilizing activity (Goldstein and Liu 1987; Liu et al. 1992). It was shown that 60 mM gluconic acid resulted in the release of approximately 0.1 mM inorganic phosphate (Pi) and it was suggested that gluconic acid produced may cause the release of protons that finally solubilized the insoluble P (Goldstein 1995). The gluconic acid so produced may further oxidized to 2-keto gluconic acid, a very strong naturally occurring organic acid (pK_a—2.6). Thus, mineral phosphate solubilization phenotype is the result of gluconic and 2-keto gluconic acid production via the direct oxidation pathway involving enzymes located on the outer face of the cytoplasmic membrane. The enzymes include glucose dehydrogenases (GDH) that oxidize glucose to gluconic acid (Goldstein 1996) and the cofactor, pyrrolquinoline quinone (PQQ). It was proposed that direct glucose oxidation to gluconic acid is a major mechanism for mineral phosphate solubilization in Gram-negative bacteria.

Production of carboxylic anions is another important mechanism for phosphate mobilization by rhizosphere bacteria. Ryan et al. (2001) reported that among the carboxylic acids identified, dicarboxylic (oxalic, tartaric, malic, fumaric, malonic acids) and tricarboxylic (citric) acids are more effective for P mobilization. Thus, phosphate solubilization/mobilizing effect of microorganisms is due to a combined effect of pH and carboxylates (Puente et al. 2004; Rodriguez et al. 2006). Otani et al. (1996) reported that carboxylic anions are able to replace phosphate from sorption complexes by ligand exchange. Under acidic soil pH conditions, the phosphate ions are precipitated by Fe^{3+} and Al^{3+} and organic acids prevent such precipitation by chelation, forming metalo-organic molecules, e.g., ferric citrate by citric acid (Mortensen 1963). The chelation by dibasic acids may also lead to ion

Table 11.1 Organic acids produced by some Gram-negative bacteria

Bacteria	Organic acids produced	References
Acetobacter sp.	Gluconic acid	Galar and Bolardi (1995)
Azospirillum sp.	Gluconic acid	Rodriguez et al. (2004)
Enterobacter sp.	Malic, gluconic acid	Shahid et al. (2012)
Escherichia freundii	Lactic acid	Sperber (1958)
Pantoea eucalypti	Gluconic acid	Castagno et al. (2011)
Pseudomonas sp.	Gluconic acid	Illmer and Schinner (1992)
Pseudomonas sp.	Citric, gluconic acid	Taha et al. (1969)
Pseudomonas aeruginosa	Gluconic acid	van Schie et al. (1985)
Pseudomonas fluorescens	Gluconic acid, malic, succinic, lactic, fumaric, tartaric, and transaconitic acid	Henri et al. (2008)
Pseudomonas striata	Tartaric, citric acid	Gaur (1990)
Rhizobium leguminosarum	2-ketogluconic acid	Halder et al. (1991)
Sinorhizobium meliloti	Malic, succinic, fumaric acid	Bianco and Defez (2010)

exchanges with hydroxyl phosphates, forming hydroxyl salts of Fe and Al releasing the phosphate ions. Citrate has also been reported to release P from goethite (Geelhoed et al. 1999) or amorphous ferric hydroxides (Dye 1995). Oxalate was also found very effective but was not produced in sufficient amounts by the PSB strains tested. In general, the ability of different carboxylic anions to desorb P decreases with a decrease in the stability constants of Fe or Al-organic acid complex in the order: citrate > oxalate > malonate/malate > tartrate > lactate > gluconate > acetate > formate (Ryan et al. 2001). This result serves to confirm the ability of the strains tested in mobilizing P from insoluble sources, in particular those producing altogether citrate, malate, and tartarate.

Henri et al. (2008) isolated three *P. fluorescens* strains (CB501, CD511, and CE509) from acidic soils of Cameroon, having the ability to solubilize three phosphate types ($Ca_3(PO_4)_2$, $AlPO_4{\cdot}H_2O$, or $FePO_4{\cdot}2H_2O$). It was found that calcium phosphate (Ca-P) solubilization resulted from the combined effects of pH decrease and carboxylic acids synthesis. At pH 4, it was solubilized by most of the organic acids. However, the synthesis of carboxylic acids was the main mechanism involved in the process of aluminum phosphate (Al-P) and Fe-P solubilization. Both were mobilized at pH 4 by citrate, malate, tartarate, and on a much lower level by gluconate and transaconitate. Bianco and Defez (2010) reported that RD64 strain, a *Sinorhizobium meliloti* 1021 strain engineered to overproduce indole-3-acetic acid (IAA) and improved nitrogen fixation ability, was also found highly

effective in mobilizing P from insoluble sources such as phosphate rock (PR). Under P-limiting conditions, the higher level of P-mobilizing activity of RD64 than of the 1021 wild-type strain is connected with the upregulation of genes coding for the high-affinity P transport system, the induction of acid phosphatase activity, and the increased secretion into the growth medium of malic, succinic, and fumaric acids. *Medicago truncatula* plants nodulated by RD64 (*Mt*-RD64), when grown under P-deficient conditions, released larger amounts of another P-solubilizing organic acid, 2-hydroxyglutaric acid, than plants nodulated by the wild-type strain (*Mt*-1021).

In few other cases, the degree of solubilization was not necessarily correlated with acidity or with the decline in pH (Krishanaraj 1987; Asea et al. 1988). Solubilization of Ca-P has even been reported to occur even in the absence of organic acid (Illmer and Schinner 1992). An HPLC analysis of the culture suspension of *Pseudomonas* did not detect any organic acid even though the bacterium solubilized unavailable forms of P (Illmer and Schinner 1995). In each of these cases, acidification of the medium resulted and was postulated that H^+ excretion originating from NH_4 assimilation contributed to acidification (Parks et al. 1990). Krishanaraj (1996) derived MPS^- mutants from *Pseudomonas* and compared with their wild-type with respect to the Pi release in the TCP broth, drop in pH, and identification of organic acid released in the medium. It was found that a highly coordinated reaction caused the dissolution of insoluble P. In the event of P stress, glucose is utilized and gets converted to organic acids that provide H^+ and get cotransported into the external mileu with $H_2PO_4^-$ or HPO_4^{-2}. These reactions are hypothesized to involve the membrane enzymes and organic acid transporters.

11.4.1.2 Production of Inorganic Acids

The solubilization of inorganic P in some cases is attributed to the production and release of inorganic acids (Richardson 2001; Reyes et al. 2001). In the special case of ammonium- and sulfur-oxidizing chemoautotrophs, nitric acid and sulfuric acids are produced (Dugan and Lundgren 1965). The inorganic acids convert $Ca_3(PO_4)_2$ to di- and monobasic phosphates with the net result of an enhanced availability of the phosphorus to plants. Nitric or sulfuric acids produced during the oxidation of nitrogenous materials or inorganic compounds of sulfur react with RP and thereby increase the soluble P. Thus, oxidation of elemental sulfur is a simple and effective means of providing utilizable phosphates. For example, a mixture may be prepared with soil or manure, elemental sulfur, and RP. As the sulfur is oxidized to sulfuric acid by *Thiobacillus*, there is a parallel increase in acidity and net release of soluble P. Nitrification of ammonium salts also leads to a slight but significant liberation of soluble P from RP composts. However, biological sulfur or ammonium oxidation has never been adopted on a commercial scale because of the availability of cheaper and more efficient means of preparing fertilizers. Gaur (1990) observed solubilization of Mussourie rock phosphate (MRP) in soil amended with ammonium sulfate. The available P increased greatly in soil inoculated with PSM and the increase in

solubilization was more with fungal inoculation followed by bacteria and yeast. Application of 1 % farmyard manure further improved P solubilization. The structural complexicity and particle size of P and the quantity of organic acid secreted by microbes were also reported to affect P solubilization.

11.4.1.3 Other Mechanisms of Phosphate Solubilization

Although phosphate solubilization commonly requires acid production, other mechanisms may account for ferric phosphate mobilization. In flooded soil, the iron available as insoluble ferric phosphates may be reduced leading to the formation of soluble iron with concomitant release of P into solution. Such increases in the availability of P on flooding may explain why rice cultivated under water has a lower requirement for fertilizer P than the same crop grown in dry land agriculture. Phosphorus may also be made available for plant uptake by certain bacteria that librates H_2S. Fermentative microorganisms produce H_2S from sulfur-containing aminoacids, or anaerobic sulfate-reducing bacteria like *Desulphovibrio* and *Desulfatomaculum* causes reduction of sulfate to H_2S when the redox potential is low. Hydrogen sulfide reacts with ferric phosphate to yield ferrous sulfide and librates the phosphate.

Humic and fulvic acids are the other chelating substances produced during the decomposition of organic materials. Mishra et al. (1982) reported that 5 % solution of humic acid in alkali could solubilize 362 μg P per gram of RP. The action of humic and fulvic acid is due to the presence of hydroxyl, phenolic, and carboxyl groups (Banger et al. 1985). Respiratory H_2CO_3 production by plants and soil organisms has been found as an alternate mechanism of mineral phosphate solubilization (Juriank et al. 1986). The CO_2 produced in the rhizosphere due to decomposition of organic matter by microbes has also been reported to be involved in increased P availability to plants. The reaction may be with CO_2 directly or due to formation of carbonic acid which reacts with $Ca_3(PO_4)_2$ forming $CaHPO_4$ or $Ca(H_2PO_4)_2$ and $CaCO_3$. Rhizosphere acidification resulting from proton release during N_2 fixation (Tang et al. 1998; Hinsinger et al. 2003) is another process which enhances P availability in alkaline soils because the solubility of Ca phosphates increases with decreasing pH.

11.4.1.4 Isolation of Mineral Phosphate-Solubilizing (mps) Genes

The conversion of insoluble phosphates (both organic and inorganic) to a form accessible to the plants, like organophosphate, is an important trait for a plant growth promoting rhizobacteria (PGPR) for increasing plant yields. Molecular biology techniques are an advantageous approach for obtaining and characterizing improved PGPR strains (Rodriguez and Fraga 1999; Igual et al. 2001). Introduction or overexpression of genes involved in soil P solubilization in natural rhizospheric bacteria is a very attractive approach for improving the capacity of microorganisms

to apply as inoculants. Cloning and transfer of phosphate-solubilizing genes into microorganisms that do not have this capability may avoid the current need of mixing two populations of nitrogen-fixing and phosphate-solubilizing bacteria when used as inoculants (Bashan et al. 2000).

The repression of mineral phosphate-solubilizing activity was observed in the presence of increasing levels of inorganic P in the medium. Goldstein (1986) reported the complete inhibition of MPS activity by *Erwinia herbicola* by addition of 20 mM Pi in the medium. Similarly, it was found that externally added K_2HPO_4 inhibited the MPS activity of *Pseudomonas* Psd 201 (Krishanaraj 1996). The phosphate stress induction of MPS activity and repression of MPS activity by externally added Pi indicated the physiologically regulated gene expression of MPS activity in bacteria. Based on these observations, Goldstein (1986) proposed the existence of *mps* genes in *Erwinia herbicola*. Several genes were induced under P starvation in *E. coli* and constituted the Pho regulon. Recently, the transcriptional control of Pho regulon has been extensively studied in *E. coli* (Makino et al. 2007), *Bacillus subtilis* (Huelett et al. 2007), and *Saccharomyces cerevisiae* (Ogawa et al. 2007). Gene(s) involved in mineral phosphate solubilization from Gram-negative bacteria *Erwinia herbicola* were cloned using shotgun-cloning experiments (Goldstein and Liu 1987) and GDH-mediated dissimilatory bypass system, involving direct oxidation of glucose to gluconic acid in the periplasmic space was found responsible for the mineral phosphate solubilization in *Erwinia herbicola*. Expression of the *mps* gene allowed production of GA in *E. coli* HB101 and conferred the ability to solubilize hydroxyapatite (MPS^+ phenotype). MPS^- mutants of *E. coli* can synthesize GDH, but not PQQ; thus it did not produce GA. On screening a cosmid pHC76 library from *Erwinia herbicola*, they found that a 55 kb insert DNA was able to transform *E. coli*. Transposon mutagenesis of the cosmid construct pMCG 898 carrying a 4.5 kb insert showed that the essential gene was localized in a 1.8 kb region. Based on sequence comparison and minicell analysis, Liu et al. (1992) deciphered that the gene codes for an enzyme pyrrolquinoline quinone (PQQ), a cofactor for the enzyme glucose dehydrogenase (GDH). The cloned 1.8 kb locus encoded protein was found similar to the gene III product of a *pqq* synthesis gene complex from *Acinetobacter calcoaceticus* and to *pqq*E of *Klebsiella pneumoniae* (Liu et al. 1992). Coincidentally, nucleotide sequence analysis of a 7 kb fragment from *Rhanella aquatilis* genomic DNA that induced hydroxyapatite solubilization in *E. coli*, showed two complete open reading frames (ORFs), and a partial ORF. One of the cloned proteins showed similarity to *pqq*E of *E. herbicola*, *K. pneumoniae*, and *A. calcoaceticus* (Kim et al. 1998b), while the partial ORF is similar to the *pqq*C of *Klebsiella pneumoniae*. These genes complemented the cryptic *pqq* genes in *E. coli*, thus allowing GA production.

Another type of gene (*gab*Y) involved in GA production and MPS was cloned from *Pseudomonas cepacia* (Babu-khan et al. 1995). The deduced amino acid sequence was found similar to histidine permease membrane-bound components. In the presence of *gab*Y, GA is produced only if *E. coli* strain expresses a functional glucose dehydrogenase (*gcd*) gene. It was speculated that this ORF could be related to the synthesis of PQQ by an alternative pathway or the synthesis of a *gcd* cofactor

different from PQQ (Babu-khan et al. 1995). In addition, a DNA fragment from *Serratia marcescens* induced quinoprotein glucose-mediated gluconic acid production in *E. coli*, but showed no homology to *pqq* or *gcd* genes (Krishanaraj and Goldstein 2001). They suggested that this gene acted by regulating GA production under cell-signal effects. Other isolated gene JM109 (pKKY) involved in the MPS phenotype was obtained from genomic DNA fragment of *Enterobacter agglomerans* using cosmid (pHC79) genomic library (Kim et al. 1997). The complementation of this gene in *E. coli* JM109 showed the MPS activity, although the pH of the medium was not altered. These results indicate that acid production is an important way, but not the only mechanism, of P solubilization by bacteria (Illmer and Schinner 1995). All these findings demonstrate the complexity of MPS in different bacterial strains, but at the same time, offer a basis for better understanding of phosphate solubilization process.

11.4.1.5 Manipulation of MPS Genes for PGPR Improvement

Expression of the *mps* genes from *Ranella aquatilis* in *E. coli* supported a much higher GA production and hydroxyapatite dissolution in comparison with the donor strain (Kim et al. 1998b), suggesting that different genetic regulation of the *mps* genes might occur in both species. MPS mutants of *Pseudomonas* spp. showed pleiotropic effects, with apparent involvement of regulatory *mps* loci in some of them (Krishanaraj et al. 1999). Two distinct classes of mutants namely, non-solubilizers (MPS^{-}) and delayed expression types (MPS^{d}) were obtained through nitrosoguanidine and Tn5 mutagenesis of *Pseudomonas* strain Psd 201. These mutants also showed different phenotypic classes with respect to metabolic and cell surface properties. The nature of pleiotropies shown by these mutants indicated that these mutational lesions might have occurred in some of the regulatory *mps* loci since the level of expression of zone and time of solubilization got affected in some mutants (Krishanaraj et al. 1999). Gene bank of the MPS^{+} wild-type *Pseudomonas* sp. Psd 201 was mobilized from *E. coli* into MPS^{-} derivative strain *Pseudomonas* Psd 207. Two clones were isolated which could restore MPS^{+} phenotype to Psd 207 and had an insert of the size of 11.8 kb that might contain one or more *mps* loci.

Expression of the mineral phosphate-solubilizing genes (*mps* genes) in a different host could be influenced by the genetic background of the recipient strain, the copy number of the plasmids present, and metabolic interactions. An attempt to improve MPS in PGPR strains, using a PQQ synthase gene from *E. herbicola* was carried out (Rodriguez et al. 2000b). This gene was subcloned in a broad-host range vector pKT230. The recombinant plasmid was expressed in *E. coli* and transferred to PGPR strains of *Burkholderia cepacia* and *Pseudomonas aeruginosa*, using tri-parental conjugation. Several of the exconjugants that were recovered in the selection medium showed a larger clearing halo zone in medium with tricalcium phosphate as the sole P source. This indicated that heterologous expression of this gene in the recombinant strains, gave rise to improved MPS ability in these PGPRs.

A bacterial citrate synthase gene was reported to increase exudation of organic acids and P availability to the plant when expressed in tobacco roots (Lopez-Bucio et al. 2000). Citrate overproducing plants yielded more leaf and fruit biomass when grown under P-limiting conditions and required less P fertilizer to achieve optimal growth. This shows the putative role of organic acid synthesis genes in P uptake in plants.

11.4.2 Mineralization of Organic Phosphorus

The chief source of organic phosphorus compounds entering the soil is the vast quantity of vegetation that undergoes decay. Agricultural crops commonly contain 0.05–0.5 % P in their tissues and this element is found in several compounds or groups of substances in plants, i.e., phytin, phospholipids, nucleic acids, phosphorylated sugars, coenzymes, and related compounds. Phosphorus may also be present as inorganic orthophosphate, especially in vacuoles and internal buffers. The phosphorus in phytin, phospholipids, and nucleic acids is found as phosphate. The nucleic acids, RNA and DNA, consist of a number of purine and pyrimidine bases, pentose sugar, and phosphate. In bacterial cell, the bulk of P is in RNA, usually accounting for one-third to somewhat more than one-half of all the P. DNA contributes from 2 to 10 % of the total P content. The acid-soluble fraction of bacterial protoplasm contains ortho- and metaphosphate, sugar phosphates, many of the coenzymes, and adenosine phosphates.

In this process of organic phosphate solubilization, microorganisms convert the organic P to inorganic forms (Deubel et al. 2000). Thus, the bound element in the plant residue material and in soil organic matter is made available to succeeding populations of plants by the action of bacteria, fungi, and actinomycetes. The mineralization and immobilization of this element are related to the analogous reactions of nitrogen. As a rule, phosphate release is most rapid under conditions favoring ammonification (nitrogen mineralization). Thus, a highly significant correlation is observed between the rates of N and P conversion to inorganic forms and the nitrogen mineralized being from 8 to 15 times, the amount of phosphate made available. There is also a correlation between C (CO_2 release) and P mineralization (a ratio of 100 to 300:1). The results showed that the ratio of C:N:P mineralized microbiologically at the equilibrium condition is similar to the ratios of three elements in humus. Gross organic P mineralization under steady-state conditions can be quantified using isotopic dilution techniques (Achat et al. 2009a, b; Bünemann et al. 2007; Oehl et al. 2001). However, the biological processes of microbial immobilization, remineralization of immobilized P, and mineralization of nonmicrobial organic P likewise replenish phosphate ions in the soil solution (Frossard et al. 2000).

Phosphorus can be released from organic compounds in soil by three groups of enzymes: (1) nonspecific phosphatases, which perform dephosphorylation of phosphor ester or phosphor anhydride bonds in organic matter; (2) phytases, which

specifically cause P release from phytic acid; and (3) phosphonatases and C-P lyases enzymes that perform C-P cleavage in organophosphonates. The main activity apparently corresponds to the work of acid phosphatases and phytases because of the predominant presence of their substrates in soil. Availability of organic phosphate compounds for plant nutrition could be a limitation in some soils resulting from precipitation with soil particle ions. Therefore, the capability of enzymes to perform the desired function in the rhizosphere is a crucial aspect for their effectiveness in plant nutrition.

11.4.2.1 Nonspecific Acid Phosphatases

Utilization of organic P by plants and microorganisms requires mineralization (hydrolysis) of phosphorus-containing substrates by phosphatase enzymes which may be of either plant or microbial origin. In plants, this process includes the release from roots of extracellular phosphatases that are considered to be important for capture and recycling of organic P lost from roots or to allow greater access to soil organic P (Richardson et al. 2005). Enhanced phosphatase activity in the rhizosphere in response to P deficiency has been observed across a wide range of plant species and is commonly reported to be higher in P-deficient soils. Chen et al. (2002) showed that depletion of soil organic P was associated with a significant increase in the activity of both mono- and diester phosphatases.

Soil microorganisms produce a range of phosphatases when cultured in laboratory media and have the capacity to utilize P from various forms of organic P that occur in soil. This includes inositol phosphates (phytate and *myo*-inositol hexakisphosphate along with other isomers) and a predominant form of organic P identified in many soils (Lim et al. 2007; Turner 2007). When added to soils, organic P substrates (both mono- and diester) are rapidly hydrolyzed (Macklon et al. 1997). Conversely, when soil suspensions or soil extracts are treated with an excess of phosphatase activity, appreciable amounts of orthophosphate can be released (George et al. 2007). Bünemann (2008) reported that upto 60 % of the total organic P may typically be hydrolyzed by phosphatases with highest amounts being released by phytases (monoester phosphatases active against phytate). Both plant and microbial phosphatases are effective in releasing orthophosphate from soil organic P, with some evidence that microbial enzymes show higher efficiency for P release (Tarafdar et al. 2001). Increased mineralization of soil organic matter associated with higher microbial activity also occurs in the rhizosphere as a result of a microbial "priming effect" due to utilization of exudate C with subsequent mineralization of nutrients from soil organic matter (Cheng 2009).

A single phosphatase enzyme may catalyze the cleavage of ethyl phosphate, glycerophosphate, and phenyl phosphate. On the other hand, diesters may require different enzymes for their breakdown. Phosphatases acting on phospholipids and nucleic acids have diesters as their substrates. The phosphatase enzyme catalyzing hydrolysis of the monoesters often has distinct optima in pH for maximum activity, i.e., active at low pH ranges are acid phosphatases, whereas the enzymes active at

high pH ranges are termed as alkaline phosphatases. Bacterial nonspecific acid phosphatases (phosphohydrolases) (NSAPs) are formed by three molecular families, which have been designated as molecular class A, B, and C (Thallar et al. 1995a). From their cellular location, these enzymes seem to function as organic phosphoester scavengers, releasing inorganic phosphates from nucleotides and sugar phosphates, and thus providing the cell with essential nutrients (Beacham 1980; Wanner 1996).

Several genes involved in biosynthesis of acid phosphatase in Gram-negative bacteria have been characterized (Rossolini et al. 1998). These cloned genes encoding acid phosphatase represent an important source of material for genetic transfer to PGPR strains. For example, the *acp*A gene isolated from *Francisella tularensis* expresses an acid phosphatase with optimum action at pH 6 and with a wide range of substrate specificity (Reilly et al. 1996). Also, genes encoding nonspecific acid phosphatases class A (PhoC) and class B (NapA) isolated from *Morganella morganii* are very promising, since the biophysical and functional properties of the encoded enzymes were extensively studied (Thallar et al. 1994, 1995b). Besides, they are P-irrepressible enzymes showing broad substrate action and high activity around pH 6 and at 30 °C. Macaskie et al. (1997) reported on the successful use of class A NSAPs as tools for environmental bioremediation of uranium-bearing waste water and on heavy metal biomineralization, particularly nickel (Bonthrone et al. 1996; Baskanova and Macaskie 1997). Moreover, the transfer and expression of these genes encoding for NSAPs into plant growth-promoting rhizobacteria could result in bacterial strains with improved phosphate-solubilizing activity. Rodriguez et al. (2000a) isolated a gene from *Burkholderia cepacia* that facilitates phosphatase activity. This gene codes for an outer membrane protein that enhanced the synthesis of soluble phosphates in the medium and could be involved in P transport to the cell. Rodriguez et al. (2006) constructed a plasmid for the stable chromosomal insertion of the *pho*C phosphatase gene from *Morganella morganii* using the delivery system developed by Lorenzo et al. (1990). This plasmid was transferred to *Azospirillum* spp. and the strains with increased phosphatase activity were obtained. Two nonspecific periplasmic acid phosphatase genes (*nap*D and *nap*E) were cloned from *Rhizobium meliloti* (Deng et al. 1998, 2001). The *nap*A phosphatase gene from the soil bacterium *Morganella morganii* was transferred to *Burkholderia cepacia* IS-16, a strain used as biofertilizer, using the broad host range vector PRK293 (Fraga et al. 2001). An increase in extracellular phosphatase activity of the recombinant strain was achieved.

11.4.2.2 Phytases

Phytate is the major component of organic forms of P in soil (Richardson 1994). Phytate is the primary source of inositol in its basic form and the major stored form of phosphate in plant seeds and pollen. Monogastric animals are incapable of using the P bound in the phytate because their gastrointestinal tracts have low levels of

phytase activity. Thus, nearly all the dietary phytate phosphorus ingested by these species is excreted, resulting in P pollution in areas of intensive animal production. Supplemental microbial phytase in corn–soybean meal diets for swine and poultry effectively improved phytate phosphorus utilization by these animals and reduced their fecal P excretion by up to 50 % (Lei et al. 1993). Therefore, phytases have emerged as very attractive enzymes for industrial and environmental applications. Most phytases belong to high molecular weight acid phosphatases. The phytase enzyme librates phosphate from phytic acid or its calcium–magnesium salt (phytin) resulting in accumulation of inositol. Some species make intracellular phytase, while others excrete extracellular phytase enzymes. Moreover, some phytases are reasonably specific and act chiefly on inositol phosphates, whereas nonspecific phosphatases remove phosphorus from dissimilar organic compounds. Phytase activity is widespread and about 30–50 % of the bacterial isolates from soil synthesized this enzyme. Its activity in nature is enhanced by addition of carbonaceous materials that increase the size of community. Species of *Aspergillus*, *Rhizopus*, *Cunninghamella*, *Arthrobacter*, *Streptomyces*, *Pseudomonas,* and *Bacillus* have been found to synthesize the phytase enzyme.

The ability of plants to obtain P directly from phytate is very limited. However, the growth and P nutrition of *Arabidopsis* plants supplied with phytate was improved significantly when they were genetically transformed with the phytase gene (*phy*A) from *Aspergillus niger* (Richardson et al. 2001a). This resulted in improved P nutrition such that the growth and P content of the plant was equivalent to control plants supplied with inorganic P. The enhanced utilization of inositol phosphate by plants in the presence of soil microbes has also been reported (Richardson et al. 2001b). Therefore, developing agriculture inoculants with high phytase production would be of great interest for improving plant nutrition and reducing P pollution in soil.

Thermally stable phytase gene (*phy*) from *Bacillus* sp. DS11 (Kim et al. 1998d) and from *B. subtilis* VTT E-68013 (Kerovuo et al. 1998) have been cloned. Han et al. (1999) reported that 1.4 kb DNA fragment containing the coding region of the *phy*A gene from *Aspergillus niger* was expressed in *Saccharomyces cerevisiae*. The recombinant extracellular phytase from *S. cerevisiae* effectively hydrolyzed phytate phosphorus from corn or soybean meal in vitro. Acid phosphatase phytase genes from *E. coli* (*app*A and *app*A2 genes) have also been isolated and characterized (Rodriguez et al. 1999; Golovan et al. 2000). The bifunctionality of these enzymes makes them attractive for solubilization of organic P in soil. Richardson et al. (2001a) showed that when grown in defined media, utilization of phytate-P by grass and legume pasture species was improved by inoculation of bacterial isolate with high phytase activity. Also, neutral phytases have great potential for genetic improvement of plant growth-promoting rhizobacteria. Neutral phytase genes have been cloned from *B. subtilis* and *B. licheniformis* (Tye et al. 2002). For example, a *phy*A gene was cloned from the FZB45 strain of *B. amyloliquefaciens*, having plant growth promoting activity (Idriss et al. 2002). It showed the highest extracellular phytase activity and the diluted culture filtrates of these strains stimulated growth of maize seedlings under limited P in the presence of phytate. Culture filtrates

obtained from a phytase negative mutant strain, whose *phy*A gene was disrupted, did not stimulate plant growth. In addition, growth of maize seedlings was enhanced in the presence of purified phytase.

Plants genetically modified to release an extracellular fungal phytase (from *Aspergillus niger*) from roots showed similar novel ability to acquire P directly from phytate (Richardson et al. 2005). Assessment of rhizosphere soils after plant growth indicated a depletion of phytase-labile P that, although soil-type dependent, did not differ substantially between control and transgenic lines or to control soils without plants (Richardson et al. 2009b). This suggests that 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 plant's inability to otherwise acquire P directly from phytate. Thus, these experiments provided strong evidence that phytase activity can be important for stimulating plant growth under limited P in soil and support the potential of using phytase genes to improve or transfer the P-solubilizing trait to PGPR strains used as agricultural inoculants.

11.5 Plant Growth Stimulation by Inoculation of Phosphate-Solubilizing Bacteria

Inoculation of crop plants with P-mineralizing microorganisms resulted in enhanced crop productivity and thus provided evidence for microbially mediated P availability to plants. Various mechanisms are employed by microorganisms to enhance the capacity of plants to acquire P from soil including (1) increased root growth through hormonal stimulation of root growth by production of indole-3-acetic acid, gibberellins, or ACC deaminase enzyme (Richardson et al. 2009a; Malik and Sindhu 2011; Khandelwal and Sindhu 2012); (2) alteration of sorption equilibria that may result in increased net transfer of orthophosphate ions into soil solution or facilitate the mobility of organic P either directly or indirectly through microbial turnover (Seeling and Zasoski 1993); and (3) through induction of metabolic processes that are effective in directly solubilizing and mineralizing P from sparingly available forms of soil inorganic and organic P (Richardson et al. 2009a).

Inoculation of cereal or legume plants with different P-solubilizing microorganisms generally resulted in improved growth and P nutrition, especially under glasshouse conditions and in fewer cases under the field conditions (e.g., see reviews by Kucey et al. 1989; Rodriguez and Fraga 1999; Gyaneshwar et al. 2002; Sindhu et al. 2009; Zaidi et al. 2009; Khan et al. 2010). In some cases, inconsistent performance was observed under field conditions and it was commonly attributed to various factors that include lack of persistence and competiveness of introduced microorganisms in soil and poor understanding of actual mechanisms involved in growth promotion, where P-mobilization may not

necessarily be the primary mechanism (Sindhu and Dadarwal 2000; Richardson 2001; Zaidi et al. 2009).

11.5.1 Inoculation Effect of P-Solubilizing Bacteria on Crop Growth

The first evidence to show that inoculation of seedling with P-solubilizing bacteria increased the P uptake and yield of oat was performed by Gerretson (1948). Subsequently, improved plant growth responses and increased Pi uptake on addition of RP were reported (Banik and Dey 1983b; Bagyaraj et al. 2000; Sindhu et al. 2010). Phosphatic biofertilizers were first prepared in USSR using *Bacillus megaterium* var. *phosphaticum* as P-solubilizing bacteria and the product was named as "phosphobacterin." It was extensively used in collective farming for seed and soil inoculation to cover an area of 14 million hectares annually and reported to give 5–10 % increase in crop yields. Inoculation experiments conducted with phosphobacterin and other PSM for various crops like oat, wheat, potatoes, groundnut, peas, soybean, tomatoes, and tobacco showed an average 10–15 % increase in yields in about 30 % of the experiments conducted (Kundu and Gaur 1980a; Agasimani et al. 1994; Dubey 1997). The variations under field conditions are expected due to the effect of various environmental conditions and survival of the inoculant strains in the soil.

The agronomic influence of some commonly used phosphorus-solubilizing bacterial species is listed in Table 11.2. Inoculation of phosphorus-solubilizing bacteria along with RP resulted in increased availability of Pi for plant utilization (Hebbara and Suseeladevi 1990; Jisha and Alagawadi 1996). It was observed that inoculation of mineral phosphate-solubilizing bacteria (MPSB) along with application of 17.5 kg P ha^{-1} as Mussoorie rock phosphate (MRP) resulted in increased dry matter in chickpea and was as effective as single super phosphate application (Prabhakar and Saraf 1990). Kundu and Gaur (1984) observed positive effect on inoculation with a mixture of *Pseudomonas striata* and *Aspergillus awamorii* in rice crop. Increase in dry matter production and P uptake from 10 to 27 % and 15 to 34 %, respectively, was observed by inoculation of *Penicillium bilaji* in chernozemic soil with low P availability in wheat crop (Kucey 1987, 1988). The addition of RP (low P solubility) had little effect, while monoammonium phosphate (commercial fertilizer with high soluble P content) resulted in the highest yields and P uptake. The addition of *P. bilaji* to these P sources did not increase P availability but increased release of P from soil (Kucey 1987). Rachewad et al. (1992) reported that addition of PSB along with RP resulted in increased P uptake by sunflower under field conditions.de Freitas et al. (1997) observed that inoculation with PSB significantly increased the number and weight of pods and seed yield of canola (*Brassica napus*) but did not affect the P uptake. Saraf et al. (1997) showed that PSB inoculation increased seed yield (10.3 q ha^{-1}) of chickpea as compared to control

Table 11.2 Inoculation effect of phosphate-solubilizing bacteria on P uptake and crop yield

Bacteria	Crop	Conditions	Response	References
Pseudomonas putida	Canola	Greenhouse	Increased P uptake and yield	Lifschitz et al. (1987)
Pseudomonas sp.	Chickpea	Greenhouse	Increased P uptake and dry matter	Krishanaraj (1996)
Pseudomonas striata	Groundnut	Field	High pod yield and P uptake	Agasimani et al. (1994)
Bacillus subtilis, *B. circulans*, and *Aspergillus niger*	Mungbean	Field	Enhanced nodulation and grain yield	Gaind and Gaur (1991)
Enterobacter cloacae, *Burkholderia cepacia*, and *Serratia marcescens*	Bamboo	Greenhouse	Increased dry matter	Maheshkumar (1997)
Pseudomonas fluorescens	Maize	Greenhouse	Increased grain yield and P content	Henri et al. (2008)
Pseudomonas striata	Rice	Greenhouse	Increased P uptake and yield	Monod et al. (1989)
Pseudomonas striata	Soybean	Field	Increased yield and P content	Dubey (1997)
Pseudomonas striata and *Bacillus polymyxa*	Wheat	Greenhouse	Increased P uptake and yield	Kundu and Gaur (1980b)
Azospirillum lipoferum and *Bacillus megaterium*	Wheat	Greenhouse	Increased shoot P and shoot weight	El Komy (2005)
Bacillus spp. PSB9 and PSB16	Rice	Glass house	Increased P uptake in plants and higher plant biomass	Panhwar et al. (2011)
Bacillus sp.	Cotton	Field	Increased soil P and higher seed cotton yield	Qureshi et al. (2012)

(8.8 q ha^{-1}). Increased grain yield (13–69 %) and uptake of N and P was reported in chickpea by inoculation of PSB along with phosphatic fertilizers. Similarly, the grain and straw yield of chickpea was enhanced with increasing level of P (0–60 kg P_2O_5 ha^{-1}), which was further improved by inoculation of PSB (Sarawgi et al. 1999, 2000). Significantly higher yield (19.5 q ha^{-1}) was observed in soybean on PSB inoculation and on addition of 26.4 kg P ha^{-1} single super phosphate (SSP) as compared to control (16.3 q ha^{-1}) (Dubey 2001). Sharma (2003) observed that addition of RP with PSB increased grain yield (0.9–1.8 t ha^{-1}), N uptake (18–38 kg ha^{-1}), P uptake (2.7–6.6 kg ha^{-1}), and K- uptake (16–41 kg ha^{-1}) in rice–wheat cropping system.

Dey et al. (2004) found that inoculation of peanut with plant growth-promoting fluorescent pseudomonad isolate PGPR1, which solubilized TCP under in vitro conditions, significantly enhanced the pod yield (23–26 %, respectively), haulm yield, and nodule dry weight over the control during 3 years in field trials. Henri et al. (2008) conducted a greenhouse trial in *Zea mays* by inoculation of three *Pseudomonas fluorescens* strains (CB501, CD511, and CE509), having the ability

to solubilize the three phosphorus types. Inoculation of *P. fluorescens* strains showed positive effects on the growth, grain yield, and P uptake. The results revealed that strain CB501 was the best plant growth promoter with a global effect of +37 %, followed by strain CE509 (+21.2 %) and strain CD511 (+16.7 %). Thus, inoculation with phosphate-solubilizing *P. fluorescens* strains made more soluble P available to the growing maize plants. Bianco and Defez (2010) found that *Medicago truncatula* plants inoculated with P-mobilizing *Sinorhizobium meliloti* strain *Mt*-RD64 exhibited higher levels of dry-weight production than *Sinorhizobium meliloti*-1021 plants. P-starved *Mt*-RD64 inoculated plants showed significant increases in both shoot and root fresh weights when compared to P-starved *Sinorhizobium meliloti*-1021 plants. Ekin (2010) evaluated the effect of application of PSB *Bacillus* M-13, with and without varying amounts of phosphorus (P) fertilizer, on growth and yield of sunflower under field conditions. The PSB application was able to mobilize P efficiently in the sunflower and improved seed quality and oil yield. It also enhanced the head diameter, 1,000 seed weight, kernel ratio, and oil content and led to seed and oil yield increases of 15.0 and 24.7 % over no application, respectively. A much greater effect was observed when PSB was used in conjunction with P fertilizers. It was found that the highest seed yield of sunflower was achieved with about 50 kg P_2O_5 ha^{-1} when used in conjunction with PSB.

Inoculation of phosphate-solubilizing *Pantoea eucalypti* strains onto *Lotus tenuis* plants showed a significant plant growth-promoting activity (Castagno et al. 2011). Panhwar et al. (2011) evaluated the ability of two PSB strains, *Bacillus* spp. PSB9 and PSB16 on growth of aerobic rice (*Oryza sativa* L.) along with different doses of RP (0, 30 and 60 kg ha^{-1}) in glasshouse experiments. The PSB strains PSB9 and PSB16 solubilized significantly high amounts of P (20.05–24.08 mg kg^{-1}) compared to non-inoculated (19–23.10 mg kg^{-1}) treatments planted in plastic pots containing 3 kg soil. Significantly higher P solubilization (24.08 mg kg^{-1}) and plant P uptake (5.31 mg $plant^{-1}$) was observed with the PSB16 strain at the highest P level of 60 kg ha^{-1}. The higher amounts of soluble P in the soil solution increased P uptake in plants and resulted in higher plant biomass (21.48 g $plant^{-1}$) at 60 days of growth. PSB strains also increased plant height (80 cm) and improved root morphology in aerobic rice. Yousefi et al. (2011) performed the field experiment that included four soil types (clay, clay loam, loam, and sandy loam), three phosphorus fertilizer levels (0, 20, and 40 mg kg^{-1}), and four levels of phosphate-solubilizing microorganisms (PSM). Resulted indicated that the highest shoot dry matter was found in clay loam soil (21.5 g pot^{-1}) at the time of physiological maturity. Combined application of PSB and arbuscular mycorrhizal fungi (AMF) increased shoot dry matter yield, seed grain spike number, and grain yield by 52, 19, and 26 %, respectively, compared to the controls.

Chookietwattana and Maneewan (2012) observed that inoculation with halotolerant PSB *Bacillus megaterium* strain on tomato (*Lycopersicon esculentum* Mill cv. Seeda) significantly increased the germination percentage and germination index, especially at NaCl concentration between 30 and 90 mM and increased the seedling dry weight at NaCl concentration upto 120 mM. Singh et al. (2012) found

that seed inoculation of *Macrotyloma uniflorum* (horsegram) by phosphate-solubilizing *Chryseobacterium* sp. PSR10 strain showed better plant growth promotion in sterilized and unsterilized soil under greenhouse conditions. Seed inoculation in a field experiment with 50 % of the recommended dose of nitrogen and phosphorus fertilizers increased the plant growth, chlorophyll content, nitrate reductase activity, phosphorus content, and crop yield. Shahid et al. (2012) showed that inoculation of sunflower with *Enterobacter* sp. Fs-11 and its rifampicin-resistant derivative in sterile sand and natural soil resulted in increased plant height, fresh weight, dry weight, and total phosphorus contents as compared to uninoculated plants. Qureshi et al. (2012) reported that inoculation of cotton with P solubilizer *Bacillus* sp. produced significantly higher seed cotton yield 1,630 as compared to 1,511 kg ha^{-1} under field conditions in clay loam soil with pH 8.3. The highest seed cotton yield was observed at highest fertilizer level, i.e., 1,733 kg ha^{-1} with inoculum. The physical parameters like plant height, number of bolls per plant, boll weight, and soil available P were also found higher in the inoculated treatments.

11.5.2 Coinoculation of P-Solubilizing Bacteria with Other Beneficial Microbes

Several experiments conducted in legume and nonlegume crops by coinoculation of PSM with diazotrophs have shown synergistic effects with regard to increase in population of both bacteria and significant increase in crop yields in comparison to single inoculation (Kucey et al. 1989). The synergistic effect was observed after coinoculation of nitrogen-fixing bacteria with PSB. For example, the inoculation of phosphate-solubilizing bacteria either alone or in combination with *A. chroococcum* enhanced the yield and nutrient uptake of cotton and wheat in field trials (Kundu and Gaur 1980c, 1982). Increased phosphorus availability by *P. putida* to common bean plants on coinoculation with *Rhizobium phaseoli* has been found to increase nodulation of common bean (Grimes and Mount 1984). Seed inoculation with thermo-tolerant PSM (viz. *Bacillus subtilis*, *B. circulans*, and *Aspergillus niger*) improved nodulation, available P_2O_5 content of soil, root and shoot biomass, straw and grain yield, and P and N uptake by mungbean (Gaind and Gaur 1991). Soybean seeds inoculated with *Bradyrhizobium japonicum* along with inoculation of PSB showed significantly higher nodulation and yield (Chandra et al. 1995).

Increased nodulation, yield attributes, seed index, and seed yield have also been reported due to combined inoculation of *P. striata* and *B. japonicum* (Dubey 1997; Kumrawat et al. 1997). Similarly, significant increase in nitrogenase activity, growth, and grain yield of pea was found due to dual inoculation of *Rhizobium leguminosarum* and PSB (Srivastava et al. 1998). El Sayed (1999) observed that coinoculation of *Rhizobium leguminosarum* and P-solubilizing *Pseudomonas*

striata significantly increased the dry matter content, grain yield, and N and P uptake of lentil over the uninoculated control. Sonboir and Sarawgi (2000) reported increased nutrients uptake (N, P, and K), grain yield, and pods plant^{-1} with increasing level of P in chickpea that was further enhanced by inoculation of PSB. Jain and Singh (2003) found that *Rhizobium*, PSB, and potassium (50 kg ha^{-1}) increased P and N uptake by chickpea. Inoculation of PSB along with *Azospirillum* increased the grain and straw yield of barley by 6.1 and 9.2 % as compared to control (Yadav et al. 2004).

Mycorrhizal associations are best known to improve plant growth in nutritionally deficient soils by the stimulation of P uptake by fungal hyphae (Gianinazzi-Pearson 1996; Harrison 2005). Synergistic interaction between PSM and vesicular arbuscular mycorrhizal (VAM) fungi has been found and the positive responses were associated with low concentration of active calcium in soils. Ghosh and Poi (1990) reported improved nodulation, plant growth, P uptake, and PSM population due to combined inoculation with *Bacillus polymyxa* and *Glomus fasciculatum* in soybean, groundnut, mungbean, and lentil. Tilak et al. (1995) reported that dual inoculation with *Pseudomonas striata* and VAM fungi (*G. fasciculatum* and *G. mosseae*) significantly increased the bean yield, root biomass, and total P uptake by soybean plants over uninoculated control in alluvial sandy soils. The P-solubilizing bacteria behaved as mycorrhiza helper bacteria (MHB) because they promoted root colonization when associated with mycorrhizal fungi (Garbaye 1994). Toro et al. (1997) reported that combined inoculation of *G. intraradices* and *Bacillus subtilis* significantly increased plant biomass and N and P accumulation in onion plant tissues. The inoculated rhizobacteria released Pi from the added RP and at least 75 % of the P in dually inoculated plants was derived from the added RP. Kim et al. (1998c) observed a significantly higher soluble P concentration in tomato plants with the inoculation of PSB and AM fungi. Thus, these myco–rhizosphere interactions between bacterial and fungal plant association contributed to biogeochemical P cycling and promoted a sustainable nutrient supply to plants.

11.6 Conclusions and Future Prospects

Soil microorganisms play a pivotal role in various biogeochemical cycles and are responsible for the cycling of nutrients in the plant utilizable form (Wall and Virginia 1999; Sindhu et al. 2010; Richardson and Simpson 2011). These beneficial microbes influence the aboveground ecosystems by contributing to plant nutrition, plant health, soil structure, and soil fertility (Glick 1995; Sindhu et al. 2009). Various commercial products primarily based on microbial isolates capable of solubilizing P are widely promoted as plant growth promoting and developed as biofertilizers for extensive use in cropping systems for northern America, Australia, China, and India. For example, isolates of *Penicillium* spp., having the capacity to solubilize P under various laboratory conditions and the ability to colonize the

rhizosphere of a range of potential host plants, appeared to have high potential for development as inoculants (Kucey 1987; Wakelin et al. 2004; Harvey et al. 2009). On the other hand, in a recent evaluation of the performance of *Penicillium bilaii* inoculant on wheat crops across a range of 47 field experiments, Karamanos et al. (2010) reported no consistent benefit in terms of plant P nutrition and found no relationship between growth responses and any soil or environmental parameters, despite the majority of trials being responsive to P addition. In such cases, poor competitive ability and lack of persistence of inoculants in soils are commonly considered to be an important factor that may restrict their effectiveness (Sindhu and Dadarwal 2000; Richardson 2001). A key requirement for successful application of inoculants is the development of appropriate formulation and delivery systems to ensure survival and effective establishment of target microorganisms within the rhizosphere.

Opportunities for enhancing microbially mediated P availability in soils might be achieved by either management of existing populations of microorganisms to optimize their capacity to mobilize P or through the use of specific microbial inoculants. In addition, there is a need to better understand how soil properties and/or environmental factors may influence the efficacy or potential for P mobilization. Esberg et al. (2010) showed correlation between microbial respiration and changes in NaOH extractable P which suggested that microbial access to this fraction was greater. Moreover, stimulation of root growth or greater elongation of root hairs (Vessey and Heisinger 2001) by specific microorganisms may enhance plant P nutrition indirectly by allowing greater exploration of soil, rather than by direct increase in the availability of soil P. Moreover, microbial activity and community composition in the rhizosphere are influenced not only by availability of carbon but also by interaction with various plant- and microbially derived signal molecules (Badri et al. 2009; Bais et al. 2006). These secondary metabolites include flavonoids, phytoalexins, other antimicrobial compounds, and various phytostimulants (Xie and Yoneyama 2010) that may mimic or interfere with microbial signaling mechanisms through quorum sensing (e.g., *N*-acyl homoserine lactones; AHLs). Thus, quorum sensing has been found to play an important role in regulation of growth and function of various soil bacteria, including symbionts and some pathogens that are known to inhabit the rhizosphere (Barriuso et al. 2008; Teplitski et al. 2011).

Recently, different methods and techniques have been developed to characterize and conserve various agriculturally important microbial communities from different environments for their optimal utilization in agriculture (Kirk et al. 2004; Naik et al. 2008). Microbial communities in soil are highly diverse; bacteria alone may be represented by as many as 10^4 species per gram of soil with indications of more than one million distinct soil bacterial genomes (Torsvik et al. 2002; Gans et al. 2005). The knowledge generated on biodiversity and genetic manipulation of P-solubilizing bacteria will be useful to design strategies for use of these bacterial strains as inoculants in sustainable and organic agriculture. This includes ecological consideration of single microorganism (as inoculant) or different groups of soil microorganisms (as communities), how they interact in the rhizosphere or

within roots (endophytes), their ability to mobilize P from different soil fractions, and how soil and farm management practices influence these processes. Azziz et al. (2012) examined the abundance and diversity of phosphate-solubilizing bacteria (PSB) in a crop/pasture rotation experiment in Uruguay. In the first year of sampling, abundance of PSB was significantly higher in natural prairie (NP) and permanent pasture (PP) than in continuous cropping (CC). The percentage of PSB relative to total heterotrophic bacteria ranged between 0.18 and 13.13 %. PSB diversity also showed statistical differences among treatments, with PP populations more diverse than those present in CC. In the second year samples, no differences were found in PSB abundance or diversity. Similarly, George et al. (2009) found no differences in bacterial community structure in the rhizosphere or on the root surface (rhizoplane) of tobacco (*Nicotiana tabacum*) plants modified to release an extracellular fungal phytase as compared to control lines. By contrast, large differences in community structure occurred in response to soil treatments that were specifically implemented to modify P availability.

Thus, complex interactions in the rhizosphere between the PSB, other microorganisms, plant, and the environment are responsible for the variability observed in solubilization of bound phosphates, Pi uptake, and plant growth promotion. The inconsistency in performance of these inoculant strains is a major constraint to the wide spread use of PSB in commercial agriculture. Genetic manipulation of plants and microorganisms for key traits that are known to be associated with P-mobilization or growth promotion (George et al. 2005; Rodriguez et al. 2006), along with generation of specific mutants in key target genes for particular traits such as organic anion release in *Pseudomonas* spp. (Miller et al. 2010), could be useful for both elucidation of mechanisms and for quantifying their contribution to increased P availability in soil. Further, the efficacy of phosphate-solubilizing bacteria can be improved by developing the better cultural practices and delivery systems that favor their establishment in the rhizosphere. In near future, the biotechnological approaches used in manipulation of bacterial traits with improved efficiency of P solubilization in bacteria and their inoculation as phosphatic biofertilizer may enhance plant growth leading to improved crop productivity.

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