

Chapter 4

Effectiveness of Phosphate Solubilizing Microorganism in Increasing Plant Phosphate Uptake and Growth in Tropical Soils

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4.1 Soil Phosphate Sorption

Phosphate fixation is a serious problem for plant productivity in agricultural soils, particularly in highly weathered soils (Trollove et al. 2003; Sanchez and Uehara 1980). Sanchez and Logan (1992) estimated that the soils that exhibit high phosphate fixation capacity occupy 1,018 million ha in the tropics. In tropical America there are 659 million ha affected, 210 in Africa, and 199 in Asia. The term phosphate fixation is used to describe reactions that remove available phosphate from the soil solution into the soil solid phase (Barber 1995). There are two types of reactions (a) phosphate sorption on the surface of soil minerals and (b) phosphate precipitation by free Al^{3+} and Fe^{3+} in the soil solution (Havlin et al. 1999).

Phosphate sorption can occur in two ways: *nonspecific sorption* that consists of electrostatic attraction of phosphate ions by positive charges on the surface of a soil mineral; the *specific sorption* takes place when phosphate ions are exchanged by hydroxyl ($-\text{OH}$) groups of soil minerals forming a strong bond with the mineral (Bohn et al. 1985). The strength and extension of phosphate sorption vary widely among soils and follows the next order: andisols > ultisols > oxisols > mollisols, vertisols > histosols. Soil phosphate sorption capacity can be easily measured by isotherms (Fox and Kamprath 1970). To this purpose, an aliquot of 3 g of soil (dry basis) is transferred into centrifuge tubes. Then, 30 mL of 0.01 M CaCl_2 with graded amount of soluble phosphate and two drops of toluene are added into each tube. Then, the centrifuge tubes are shaken in a reciprocal shaker for 7 days. After that, the tubes are centrifuged at 4,000 rpm for 15 min and the supernatant is filtered through a filter paper. Soluble phosphate concentration is measured by the molybdate-blue method (Murphy and Riley 1962). The $P_{0.2}$ -value is an index of the soil

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phosphate sorption capacity, it means the amount of phosphorus required to achieve a soil solution phosphorus concentration of 0.2 mg L^{-1} , which is considered optimal for most plant crops (Fox 1979).

4.2 Phosphate Solubilizing Microorganisms

Many soil microorganisms can solubilize inorganic soil phosphate compounds, reversing the process of phosphate fixation (Gyaneshwar et al. 2002; Rao 1992). Soil bacteria capable of plant growth promotion such as the genera *Pseudomonas*, *Enterobacter*, and *Bacillus* are particularly active as phosphate solubilizers (Canbolat et al. 2006; Pandey et al. 2006; Xavier and Germida 2003; Kim et al. 1998a, b; Barea et al. 1975). Soil fungi especially those of the genus *Penicillium* and *Aspergillus* also have been demonstrated to be effective phosphate solubilizing microorganisms (PSMs) (Reddy et al. 2002; Whitelaw 2000; Kang et al. 2008). Kucey and Leggett (1989) found in Mollisols of Canada that 0.5 and 0.1% of the total population of bacteria and fungi, respectively, exhibited the ability to solubilize insoluble Pi compounds.

4.3 Mechanisms of Microbial Phosphate Solubilization

Several mechanisms have been proposed to explain the microbial solubilization of phosphate compounds. The mechanisms consist of (a) release of organic acids produced during organic carbon decomposition (Kang et al. 2002; Hameeda et al. 2006; Bar-Yosef et al. 1999); (b) excretion of protons due to NH_4^+ assimilation by microorganisms (Whitelaw 2000; Ilmer et al. 1995; Abd-Alla 1994; Asea et al. 1988; Roos and Luckner 1984; Kucey 1983); (c) formation of complexes between organic acids/anions with cations (Al^{3+} , Fe^{3+} , Ca^{2+}) (Welch et al. 2002); and (d) phosphate desorption from soil minerals (Osorio 2008). Bacteria of the genera *Nitrosomonas* and *Thiobacillus* species can also dissolve phosphate compounds by producing nitric and sulfuric acids (Azam and Memom 1996). Equally, phosphate compounds may be solubilized by carbonic acid formed as a result of organic matter decomposition (Memon 1996). In addition, Lopez-Bucio et al. (2007) reported that *Bacillus megaterium* (a known PSM) promoted plant growth and stimulated root branching of *Arabidopsis thaliana*, which can increase plant phosphate uptake. Increase in soil phosphate availability may be caused by several reactions involving microorganisms that produce organic acids (Stevenson 1986). These substances can replace or compete with phosphate ions for sorption sites.

Kim et al. (1997) found that the production of acidity was a major mechanism in the solubilization of hydroxyapatite by *Enterobacter agglomerans* under in vitro conditions. For comparison, Kim and coworkers employed citric acid, oxalic acid, lactic acid, and HCl at the same pH produced by *E. agglomerans*. They found that at

pH 4.0–4.1 (and a shaking time of 48–50 h) there were no significant differences among phosphate solubilization produced by this bacterium and that produced by citric acid, oxalic, and HCl. However, lactic acid exhibited a lower capacity for solubilizing hydroxyapatite.

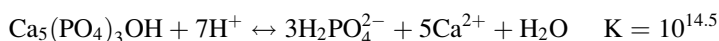
The fungus *Aspergillus niger* produced organic acids but other PSM species did not produce detectable amounts of the organic acids. Under in vitro conditions, the pH of the growth medium decreased as a result of acid production by PSMs. Osorio and Habte (2001) found an inverse relation between culture medium pH and Pi released from rock phosphate (RP) by PSMs (bacteria and fungi) isolated from Hawaiian soils. The microbial solubilization of rock phosphate was associated with the ability of microorganisms to depress the pH of the growth medium by producing organic acids.

Some of the organic acids (or their respective anions) commonly associated with microbial solubilization of phosphate are gluconic acid (Di-Simine et al. 1998; Bar-Yosef et al. 1999), oxalic acid (Osorio 2008), citric acid (Kim et al. 1997; Kucey and Leggett 1989), lactic acid, tartaric acid, and aspartic acid (Venkateswardu et al. 1984). These acids are products of microbial metabolism, in some cases by oxidative respiration or by fermentation of carbonaceous substrates (e.g., glucose) (Trolove et al. 2003; Jones et al. 2003; Gyaneshwar et al. 2002; Prescott et al. 1999; Mathews et al. 1999; Atlas and Bartha 1997). The reactions of phosphate solubilization are believed to occur in the rhizosphere where carbonaceous compounds are released and where the solubilized phosphate may be taken up by the root or mycorrhizal system. Lynch and Ho (2005) showed that in wheat plants up to 33–40% of the total carbon fixed by photosynthesis could be excreted into the rhizosphere; Amos and Walters (2006) estimated a value of 29% for maize. Many rhizosphere microorganisms are heterotrophs and might use these carbonaceous substrates to produce organic acids. Recently, Hameeda et al. (2006) found that the type of carbon source affected the effectiveness of rock phosphate solubilizing bacteria. For *Serratia marcescens* and *Pseudomonas* sp. the more favorable carbon source for rock phosphate solubilization followed the order, glucose > galactose > xylose > mannose = maltose > cellobiose > arabinose. No solubilization of RP was detected with the last carbon source of this series. The bacteria were capable of solubilizing rock phosphate using different kinds of composted crop residues (rice, pigeon pea, and a grass). Reyes et al. (2006) also compared the effect of the carbon source on rock phosphate solubilization and found that *Penicillium* sp. and *Azotobacter* sp. were more effective if the medium contained sucrose than dextrose.

When PSMs were inoculated in neutral or alkaline soils, the production of acids decreased rhizosphere pH, favoring the solubility of soil native calcium-phosphate and added rock phosphate (Kim et al. 1998a). These results have commonly been found in temperate-zone soils of Europe and North America (Kucey and Leggett 1989; Kucey 1983, 1987, 1988) and other countries, e.g., Egypt (Omar 1998) where calcareous soils are abundant.

Welch et al. (2002) found that organic acid/anions produced by microorganisms were capable of dissolving apatite by forming a complex with Ca either in solution and/or directly at the mineral surface. To illustrate this reaction, let us see

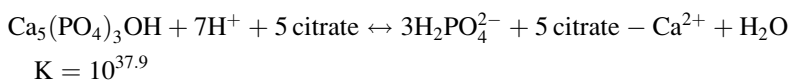
dissolution of a hydroxyapatite under acidic conditions. If proton activity increases (low pH) the reaction proceeds to right (as written):



It represents a change in free energy (ΔG) equal to $-82.72 \text{ kJ mol}^{-1}$.

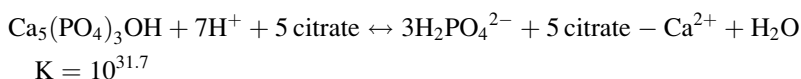
Moreover, if in the products there is also a reduction in the activity of Ca^{2+} , produced by the formation of an organic complex with Ca^{2+} (e.g., Ca^{2+} -citrate and Ca^{2+} -oxalate), the reaction will be more favorable thermodynamically ($10^{38.8}$ and $10^{31.7}$, respectively).

In the presence of citrate:



It represents a change in free energy (ΔG) equal to $-216.21 \text{ kJ mol}^{-1}$.

In the presence of oxalate:



It represents a change in free energy (ΔG) equals to $-180.73 \text{ kJ mol}^{-1}$.

Thus, the microbial solubilization of soil phosphate seems to be associated with the presence of calcium phosphates. In fact, most of the research on microbial solubilization has been done with solubilizers of tricalcium phosphate (Ca_3PO_4) (Pikovskaia 1948; Sperber 1957, 1958; Louw and Webley 1959; Agnihorti 1970; Paul and Rao 1971; Banik and Dey 1981a, b, c) or rock phosphate (Kim et al. 1998b; Osorio and Habte 2001). However, researchers on PSMs no longer recommend PSM isolation using culture medium with Ca_3PO_4 because it can supply free phosphate. Some authors reported that in vitro microbial solubilization not only occurred with calcium phosphate but also with Al and Fe phosphates. However, the solubilization was higher with calcium phosphates (Imer et al. 1995; Banik and Dey 1983; Rose 1957).

Solubilization of Al and Fe phosphates can be easily observed under in vitro conditions where no soil minerals interfere with phosphate solubility. Imer et al. (1995) found that *A. niger*, *Penicillium simplicissium*, *Pseudomonas aurantio-griseum*, and *Pseudomonas* sp. were effective in solubilizing AlPO_4 under in vitro conditions via organic acid production or proton excretion due to NH_4^+ assimilation. Aluminum and Fe ions in solution may be chelated by organic anions (e.g., oxalate and citrate) (Kang et al. 2002; Bolan et al. 1994), favoring the dissolution of Al and Fe phosphates. Whether organic acids released by PSMs can solubilize phosphate from Al and Fe phosphate under acidic soil conditions must be studied.

On the other hand, organic anions produced by PSMs also can compete with phosphate for phosphate sorption sites on the surface of soil minerals. He and Zhu

(1997, 1998) suggested that phosphate sorbed on the surfaces of some minerals was displaced when a culture medium was inoculated with soil samples containing microorganisms (unidentified) that presumably excreted organic acids. Osorio (2008) further reported that a PSM was capable of phosphate desorption from minerals and soil samples, but this was controlled by the phosphate desorption (higher phosphate desorption at low $P_{0.2}$ value). For minerals, the magnitude on which phosphate desorbed was in the order: montmorillonite > kaolinite > goethite > allophane (null desorption). Consequently, for soils the order was: mollisol > oxisol > ultisol > andisol. The amount of phosphate desorbed by the PSM was higher when the minerals or soils had higher levels of sorbed phosphate; this is when the saturation of phosphate sorption sites was higher.

4.4 Effects of PSMs on Plant Phosphate Uptake

During the 1950s–1960s, inoculation with *B. megaterium* var. phosphaticum (phosphobacterin) in Russian soils (mainly Mollisols) was the best known use of PSMs (Stevenson 1986; Kucey and Leggett 1989). At that time, the mechanisms of phosphate solubilization were not fully understood, but the mineralization of organic phosphate was proposed as the major mechanism. Trials carried out in many locations demonstrated little consistency in plant response; apparently other factors such as liming and/or organic material addition affected the effectiveness of phosphobacterin. The lack of response to phosphobacterin in many locations, pointed to possible intensified organic matter decomposition, and the poor understanding of the mechanisms of phosphate solubilization carried out by this microorganism discouraged its use. Since then, the research was oriented toward the study of microbial solubilization of inorganic phosphate compounds in the soil (Kucey and Leggett 1989).

Inoculation with PSMs has produced positive results on crop yield, plant growth, and phosphate uptake of several plant species (Kucey and Leggett 1989). For instance, some effective fungal PSMs are *A. niger* (Omar 1998; Rosendahl 1942) and *Penicillium bilaii* (Kucey 1983, 1987, 1988; Asea et al. 1988; Kucey and Leggett 1989; Gleddie 1993). Whitelaw (2000) and Kucey and Leggett (1989) reviewed the literature in the subject matter and showed several reports of increase in plant growth and phosphate uptake.

However, the effectiveness of PSMs in enhancing plant phosphate uptake has been questioned by some authors (Bolan 1991; Tinker 1980) because (a) organic substances required for these microorganisms are scarce in nonrhizospheric sites, (b) antagonism and competition by other microorganisms in the rhizosphere can reduce the effectiveness of PSMs, and (c) low translocation of solubilized phosphate through the soil because it can be refixed by soil components. This latter point is more important in soils with a high phosphate fixation capacity as discussed already.

4.5 Dual Inoculation with PSMs and AMF

Coinoculation with rhizosphere PSMs and AMF of soils with high phosphate fixation capacity may overcome the limitations mentioned on the effectiveness of PSMs in enhancing plant phosphate uptake. First, mycorrhizal plants can release higher amounts of carbonaceous substances into the rhizosphere (Linderman 1988; Rambelli 1973) than nonmycorrhizal plants. Rhizosphere PSMs can use these carbon substrates for their metabolic processes, which are responsible for organic acid production in the rhizosphere and/or proton excretion (Azcon and Barea 1996). Second, the extensive mycorrhizal hyphae network formed around roots can efficiently take up phosphate released by PSMs, thus minimizing its refixation. As long as PSMs grow in the rhizosphere (or mycorrhizosphere), there is a great opportunity to satisfy their carbon requirement and deliver phosphate into the soil solution.

Barea et al. (2002) reported that the combined inoculation with phosphate-solubilizing-rhizobacteria, mycorrhizal fungi, and *Rhizobium* increased the phosphate uptake in several legumes fertilized with rock phosphate. Some reports on the beneficial effects on dual inoculation with AMF and PSMs are presented in Table 4.1.

In tomato, beneficial results were found with *E. agglomerans* and *Glomus etunicatum* (Kim et al. 1998a) (Table 4.2). Synergistic effects have been also found in sunflower (*Helianthus annuus*) with the triple inoculation of two PSMs (*Azotobacter chroococcum* and *Penicillium glaucum*) and the AMF *G. fasciculatum* (Gururaj and

Table 4.1 Effect of PSM inoculation on plant phosphate uptake of mycorrhiza-free and mycorrhized plants grown on temperate soils

Soil type/plant	P added	PSMs	Increase of plant P uptake due to PSM inoculation (%)		References
			–AMF	+AMF	
Calcareous mixed with sand, pH 6.7 (plant: kudzu)	RP	<i>Azospirillum</i>	33	0–33	Toro et al. (1996)
		<i>Penicillium</i>	33	0–44	
		Unidentified	33	22–38	
		<i>Pseudomonas</i>	33	33–50	
Vertic Epiaqualf mixed with sand and vermiculite, pH 5.9 (plant: tomato)	RP		54 (35 days) ^a	124	Kim et al. (1998a)
			27 (55 days)	27	
		<i>Enterobacter agglomerans</i>	8 (75 days)	11	
		<i>Bacillus</i>	26	52	
		<i>Cladosporium</i>	47	69	
Sandy soil pH 7.6 (plant: wheat)	RP	<i>Bacillus + Cladosporium</i>	73	98	Singh and Kapoor (1999)
		<i>Bacillus</i>	–	248	
		<i>Cladosporium</i>	–	301	
		<i>Bacillus + Cladosporium</i>	51	344	

RP rock phosphate, ^aDays after planting

Table 4.2 Effects of *E. agglomerans* (PSM) and *G. etunicatum* (AMF) inoculation on tomato plant growth and phosphate uptake 75 days after inoculation

Treatment	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Shoot phosphate content (mg/plant)
Control	42.2 (100) ^a	4.3 (100)	116.6 (100)
PSM	48.5 (115)	5.1 (118)	125.3 (107)
AMF	47.6 (113)	5.6 (130)	120.9 (104)
PSM + AMF	54.6 (129)	6.8 (158)	134.4 (115)
LSD ($P \leq 0.05$)	1.96	0.5	9.8

^aIn parenthesis percentage of the control. Source: Kim et al. (1998a)

Mallikarjunaiah 1995). Similar effects were found in cotton with the inoculation of *Pseudomonas striata* and *Azospirillum* sp. (PSMs) and *G. fasciculatum* (AMF) (Prathibha et al. 1995). In rice, favorable effects were also reported with *P. striata* (PSM), *Bacillus polymyxa* (PSM), and *G. fasciculatum* (AMF) (Mohod et al. 1991). In chili (*Capsicum annum*) synergistic effects were reported with two AMF, *G. fasciculatum* or *G. macrocarpum*, and a PSM *P. striata* (Sreenivasa and Krishnaraj 1992). Moreover, positive results have been obtained in wheat with several combinations that include *P. striata* (PSM) and *G. fasciculatum* (AMF), *P. putida*, *P. aeruginosa* and *P. fluorescens* (PSMs) with *G. clarum* (AMF), *P. striata* and *Agrobacterium radiobacter* (PSMs) combined with two AMF, *G. fasciculatum* and *Gigaspora margarita* (Gaur et al. 1990).

Kopler et al. (1988) indicated that more legume nodulation was obtained with concurrent inoculation of *Rhizobium* and *Pseudomonas* spp. (PSMs). Sturz et al. (1997) found that nodulation by *Rhizobium leguminosarum* b.v. trifolii of red clover (*Trifolium pratense*) was promoted when it was coinoculated with the PSM *Bacillus insolitus*, *B. brevis*, or *Agrobacterium rhizogenes*. Similar results were obtained with the inoculation of *G. mosseae* (AMF) and *Azorhizobium caulinodans* (PSMs) in *Sesbania rostrata* (Rahman and Parsons 1997). In soybean, the combination of *Bradyrhizobium japonicum* (N₂ fixer) with *P. fluorescens* (PSMs) and *G. mosseae* (AMF) has given equally good results (Shabayey et al. 1996). Such results are likely to be due to a higher plant phosphate uptake promoted by the combined action of PSM and AMF, which may satisfy the high phosphate requirements of the N₂-fixing process (Azcon and Barea 1996; Young et al. 1990).

Peix et al. (2001) found that the N₂-fixing bacterium *Mesorhizobium mediterraneum* was able to solubilize Ca₃(PO₄)₂ under in vitro and soil conditions. Inoculation with *M. mediterraneum* of seeds of the chickpea (*Cicer arietinum*) and barley (*Hordeum vulgare*) planted in a Calcic Rhodoxeralf significantly increased plant growth and total nitrogen and phosphate content in both plants. Further increase was observed when *M. mediterraneum* and Ca₃PO₄ were concurrently applied. Benefits of this bacterium were not only due to the symbiotic N₂ fixation when associated with chickpea but also due to the enhancement of plant phosphate uptake of both plants.

Apparently, there is a certain degree of specificity among PSM, AMF, and phosphate source. Toro et al. (1996) studied the combined effect of AMF (*Glomus*

spp.) and eight PSMs (bacteria) on plant growth and phosphate nutrition of the tropical legume kudzu (*Pueraria phaseoloides*). The PSMs were isolated from an Oxisol and were characterized by their ability to solubilize rock phosphate, Al, and Fe phosphate compounds. In general, PSM inoculation of the kudzu–*Rhizobium*–AMF association increased plant growth, yield, and nutritional status. However, this interaction was not observed in all combinations of AMF + PSM. For instance, the three PSMs *Azospirillum* sp., *Bacillus* sp., and *Enterobacter* sp. had a higher effect when they were coinoculated with *G. mosseae*. In contrast, *Pseudomonas* sp. and an unidentified isolate had a better performance when they were combined with *G. fasciculatum*. On the other hand, Fe phosphate solubilizers were more effective if they were alone, whereas Al-phosphate and rock phosphate solubilizers performed better when they were concurrently inoculated with AMF. Reasons for these differences may be due to interactions between the microorganisms such as a more effective stimulation of rapid mycorrhizal colonization, and enhancing the length, distribution, and/or survival of external fungal mycelium. Mycorrhizal fungi might differ in the amount and type of hyphal exudates released into the mycorrhizosphere. In addition, a high capacity to solubilize phosphate might stimulate plant growth and favor mycorrhizal activity.

Kucey (1987) inoculated a Mollisol (pH 7.2) of Canada with *P. bilaii*, in which either mycorrhizal or nonmycorrhizal wheat or beans were grown. In the case of wheat, mycorrhizal inoculation alone increased significant phosphate uptake (30%), but *P. bilaii* alone did not do so. However, *P. bilaii* increased phosphate uptake of mycorrhizal wheat by 10% in the unfertilized soil, but not in the soil fertilized with rock phosphate. In the case of bean, mycorrhizal inoculation alone did not increase plant phosphate uptake, but *P. bilaii* alone was able to significantly increase it by 31%. Dual inoculation did not increase phosphate uptake beyond the level obtained with *P. bilaii*. In other words, there was not synergism between both microorganisms on bean phosphate uptake in unfertilized and rock phosphate-fertilized soil. Mollisols usually exhibit a low phosphate fixation capacity. For this reason, it is not surprising that inoculation with this PSM alone increased bean phosphate uptake. Differences between wheat and bean could be due to different types and amounts of root exudates that would stimulate acid production in the rhizosphere by PSMs.

Barea et al. (1975) found that inoculation with PSMs (*Pseudomonas* + *Agrobacterium*) did not increase plant phosphate uptake of mycorrhiza-free and mycorrhizal lavender (*Lavandula spica* L. cv. Vera) and maize (*Zea mays* L.) plants grown in an unfertilized red mediterranean soil (pH 7.5, 0.01 M CaCl₂-P: 0.021 mg L⁻¹). In contrast, PSM inoculation significantly increased plant phosphate uptake of mycorrhizal maize (24%) and mycorrhiza-free lavender (42%), but not of mycorrhizal lavender in a gray-meridional soil (pH 7.6, 0.01 M CaCl₂-P: 0.008 mg L⁻¹). Maize plants achieved 75 and 95% of their maximum yield at P concentrations of 0.008 and 0.025 mg L⁻¹ (Fox 1979), indicating that maize plants (and perhaps lavender too) satisfied most of their phosphate requirements in the red mediterranean soil.

Currently, *P. bilaii* is commercially available in North America under the name of Provide™, which has been successfully tested to enhance plant phosphate uptake of some crop plants (Whitelaw 2000). Whitelaw et al. (1997) inoculated an acidic phosphate-deficient soil of Australia (pH 4.6) with *Penicillium radicum* in combination with several levels of KH_2PO_4 (added P: 0–20 kg ha⁻¹). The inoculation with this PSM increased wheat phosphate uptake by 8% in the unfertilized soil. When the fungus was inoculated in combination with phosphate fertilization, plant P increased between 2 and 28%; the increase was highest when the rate of added P was 15 kg ha⁻¹.

Young et al. (1990) found that inoculation with either PSM or AMF significantly increased peanut (*Arachis hypogea*) production in two subtropical–tropical acidic soils of Taiwan. Inoculation with either AMF or PSMs in unfertilized soils was as effective as the addition of rock phosphate alone. Inoculation with AMF or PSMs in rock phosphate-fertilized soils did not increase peanut yield above that obtained with AMF or PSM inoculation in unfertilized soils. Unfortunately, dual inoculation of AMF and PSMs was not evaluated.

In addition, Young et al. (1990) found that the responses to single or mixed inoculations with PSM and/or AMF had variable effects on plant growth of leucaena grown in three soils of Taiwan. Inoculation with PSMs was not as effective as AMF inoculation in enhancing plant growth in the soil with the lowest available phosphate level (Hinshe soil). In the Wunfun soil (also with a low soil available phosphate level), PSM inoculation was ineffective in increasing plant growth unlike AMF. In the alkaline soil containing the highest soil available phosphate (and presumably rich in calcium-phosphates), PSM inoculation alone significantly increased plant growth (40%) above the AMF inoculation effect, which did not increase growth.

Effectiveness of PSM inoculation alone to enhance plant phosphate uptake in subtropical and tropical acidic soils is relatively low and variable. The increases recorded were 8% (Whitelaw et al. 1997), 13% (Osorio and Habte 2001), and 24–25% (Young et al. 1990) compared with those reported in less-weathered soils (mostly Mollisols) of the temperate zone, where soil phosphate-fixation capacity is low. By contrast, the effectiveness of PSM inoculation in enhancing plant phosphate uptake of mycorrhizal plants grown in tropical or subtropical soils can be relatively higher compared to data reported in temperate soils.

Mycorrhizal association in combination with PSMs is often needed to obtain improvements in plant phosphate uptake in highly weathered soils, in contrast to results obtained in less-weathered soils. In these less-weathered soils that normally exhibit low soil phosphate sorption, the inoculation of PSMs alone has been enough to increase plant phosphate uptake of nonmycorrhizal plants (Peix et al. 2001; Omar 1998; Gleddie 1993; Kucey and Leggett 1989; Asea et al. 1988; Kucey 1983, 1987, 1988). Most of the soils used by these authors were mollisols, calcareous soils, or sandy soils, which are characterized by a low phosphate sorption capacity and relatively high soil Ca–phosphate content (Cross and Schlesinger 1995). Therefore, the freshly released phosphate by PSMs can remain longer in the soil solution until its absorption by the roots.

For instance, Toro et al. (1998) found that the PSM *Enterobacter* sp. alone was as effective as the mycorrhizal fungus *G. mosseae* alone in increasing twofold the plant phosphate uptake of alfalfa grown in a calcareous soil of Spain. Duponnois et al. (2006) found that the inoculation alone with the fungus *Arthrobotrys oligospora* increased the phosphate uptake and shoot dry weight of *Acacia holoserica* grown in a sandy soil of Senegal by 56% and 46%. The increase in plant phosphate uptake and growth were even higher when rock phosphate was added with the PSMs (74 and 103%, respectively). The addition of rock phosphate alone did not increase significantly plant phosphate uptake and growth. Similar results were reported by Wakelin et al. (2004a, b) on wheat with the inoculation of *P. radicum* in some sandy soils of Australia with neutral to alkaline soil reactivity. In these soils, Wakelin and coworkers observed increases in plant growth between 34 and 76%. Even higher was the effect of *Penicillium thomii* on plant phosphate uptake of mint (*Mentha piperita*) grown in a soilless medium (vermiculite–perlite) fertilized with rock phosphate (Cabello et al. 2005). In that experiment, the inoculation with the fungus increased plant phosphate content by more than threefold compared to control plants (uninoculated and unfertilized). The rock phosphate alone was ineffective. This significant increase in plant phosphate uptake is understandable given the very low phosphate sorption on these kind of substrates.

4.6 In Vitro Test to Evaluate Effectiveness of PSMs

A method to quantify PSM effectiveness in dissolving rock phosphate was developed by Osorio and Habte (2001). This consists of preparing a soluble P-free-medium without agar (a rock phosphate is used as the unique source of P) and transfer 75 mL of this into a 250-mL Erlenmeyer flask. Then, the flasks and their contents are autoclaved (120°C, 0.1 MPa, 30 min). Later, a selected microbe (bacterium or fungus) is transferred from a Petri dish with a sterile loop. The flask also can be inoculated with 1 mL of a fungal or bacterial suspension. A control uninoculated is included for comparison. The flasks are shaken continuously in an orbital shaker at 100 rpm, 28°C for 7 days.

After the incubation, pH is measured directly. An aliquot of 20 mL is transferred into a centrifuged tube and centrifuged at 5,000 rpm for 15 min. Later, the supernatant is filtered through a filter paper (2 µm) and then by a Milipore membrane (0.45 µm). Soluble phosphate concentration is determined by the molybdate-blue method (Murphy and Riley 1962). Figure 4.1 illustrates the results of an in vitro test for 32 microbes isolated from Hawaiian soils. At the end of the incubation, the uninoculated control had a medium pH 7.0, whereas the inoculated flask had lower pHs. Significant increases in solution phosphorus concentration are detected when the pH decreases below 4.7. An attempt to classify microbes in categories of effectiveness to dissolve rock phosphate as a function of final pH is as follows: ineffective when final pH is >4.7, moderately effective when pH is 4.0–4.7. It is also possible to determine phosphate concentration in the microbial

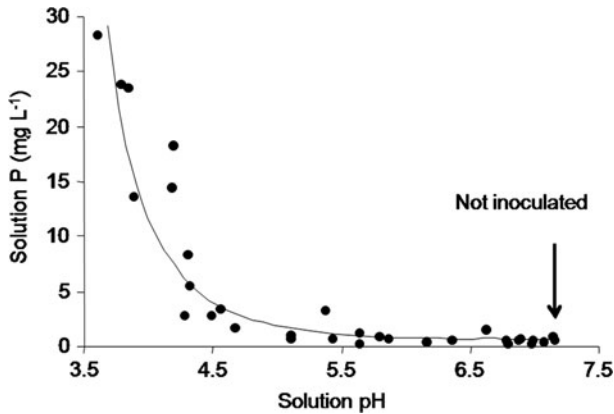


Fig. 4.1 Relationship between pH and concentration of phosphate in liquid medium inoculated or not inoculated with phosphate solubilizing microorganisms (Source: Osorio and Habte 2001)

biomass that remains at the bottom of the centrifuge tube; special care must be taken to separate the microbial biomass from the particles of rock phosphate undissolved.

4.7 In Vivo Test with Plants

In order to evaluate the effectiveness of PSMs in enhancing plant phosphate uptake and growth, a simple method has been developed. First, a sample of an autoclaved and phosphate-deficient soil is transferred into plastic pots (ca. 1 L). Then, the soil sample is amended or unamended with rock phosphate and other nutrients (based on soil test). Right before planting, the soil is inoculated or not with a PSM and a mycorrhizal fungus. In the hole of planting, author used 5–10 mL of a microbial suspension containing 10^5 – 10^6 UFC mL⁻¹ of a given PSM; 20–25 g of a crude mycorrhizal inoculum containing 40 infective propagules per gram is good enough. *Leucaena leucocephala* is our preferred indicator plant given its high phosphate requirement and its inability to absorb it in phosphate-deficient soils. After a short period of growth (60–90 days), plants are harvested and shoot dry matter and phosphate uptake is measured (Table 4.3).

In these experiments, PSM inoculation (alone) significantly increased plant growth of leucaena by 71% in the Mollisol (low phosphate sorption) and by 25% in the Oxisol de Wahiawa (HI, USA); in the other Oxisols (Quilichao and Carimagua, Colombia, medium phosphate sorption) and in the Andisol, PSM alone was ineffective in increasing plant growth. On the other hand, when the PSM was concomitantly inoculated with the AMF (AMF + PSM), the effect was significantly ($P \leq 0.05$) higher than with the AMF inoculation alone (AMF), except in the Andisol given its very high phosphate sorption capacity. In the Oxisols, the increase

Table 4.3 Shoot dry weight (g) of leucaena inoculated or not with a PSM and AMF in soils that differed in their phosphate sorption capacity

Soil	Soil phosphate sorption category	Control	PSM	AMF	AMF + PSM
Mollisol – Neira ($P_{0.2} = 45$)	Low	0.7d	1.2c	1.4b	1.5a
Oxisol – Quilichao ($P_{0.2} = 328$)	Medium	0.3c	0.3c	2.0b	2.6a
Oxisol – Carimagua ($P_{0.2} = 417$)	Medium	0.3c	0.3c	0.8b	1.0a
Oxisol – Wahiawa ($P_{0.2} = 450$)	Medium	0.4d	0.5c	1.0b	1.4a
Andisol – La Selva ($P_{0.2} = 2,222$)	Very high	0.3b	0.3b	0.5a	0.5a

Source: Osorio (2008) and Londoño (2010)

ranged from 25 to 40%. In the Mollisol, the increase was only 7%; in this case, it is likely that leucaena plants already have enough phosphate absorbed by the micorrhizal association.

4.8 Conclusions

The effectiveness of PSMs in increasing plant phosphate uptake and growth is controlled by the phosphate sorption capacity. In soils with low phosphate sorption ($P_{0.2} < 100$), PSM inoculation alone can increase plant performance. In soils with medium and high phosphate sorption ($100 < P_{0.2} < 500$; $500 < P_{0.2} < 1,000$) PSMs alone are less effective or even ineffective, their effectiveness depends on the presence of mycorrhizal association. In soils with very high phosphate sorption ($P_{0.2} > 1,000$), PSMs seem to be ineffective even in the presence of AMF.

In soils conducive for PSMs, the ability to solubilize phosphate might have some practical implications such as lowering soil phosphate fertilizer requirement and enhancing the residual effect of soluble phosphate fertilizers. Their effectiveness in increasing soil solution phosphate by dissolving rock phosphate, native soil phosphate, and/or desorbing sorbed phosphate can play an important role in the alleviation of phosphate deficiency in soils, particularly in the tropics where the high phosphate sorption capacity of soils constrains plant productivity. Moreover, the role that PSMs play in soil phosphate availability ought to be an important consideration in the development of biotechnological approaches to the managements of soils.

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