

Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure

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Received: 26 June 2012 / Revised: 16 August 2012 / Accepted: 30 August 2012 / Published online: 2 October 2012
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Abstract Literature analysis and chemical considerations of biological phosphate solubilization have shown that the commonly used selection factor for this trait, tricalcium phosphate (TCP), is relatively weak and unreliable as a universal selection factor for isolating and testing phosphate-solubilizing bacteria (PSB) for enhancing plant growth. Most publications describing isolation of PSB employed TCP. The use of TCP usually yields many (up to several thousands per study) isolates “supposedly” PSB. When these isolates are further tested for direct contribution of phosphorus to the plants, only a very few are true PSB.

This study is dedicated to the memory of the German/Spanish mycorrhizae researcher Dr. Horst Vierheilig (1964–2011) of CSIC, Spain.

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Other compounds are also tested, but on a very small scale. These phosphates (P), mainly Fe-P, Al-P, and several Ca-P, are even less soluble than TCP in water. Because soils greatly vary by pH and several chemical considerations, it appears that there is no metal-P compound that can serve as the universal selection factor for PSB. A practical approach is to use a combination of two or three metal-P compounds together or in tandem, according to the end use of these bacteria—Ca-P compounds (including rock phosphates) for alkaline soils, Fe-P and Al-P compounds for acidic soils, and phytates for soils rich in organic P. Isolates with abundant production of acids will be isolated. This approach will reduce the number of potential PSB from numerous isolates to just a few. Once a potential isolate is identified, it must be further tested for direct contribution to P plant nutrition and not necessarily to general growth promotion, as commonly done because promotion of growth, even by PSB, can be the outcome of other mechanisms. Isolates that do not comply with this general sequence of testing should not be declared as PSB.

Keywords Phosphate solubilization · Plant growth promoting bacteria · PSB

Introduction and background

Apart from the basic need for phosphorus in plant nutrition, there are three reasons why phosphate fertilization is a major agricultural research topic: (1) the price of fertilizers skyrocketed in recent times, making P fertilizers beyond the reach of many farmers in developing countries, (2)

competition for high quality rock phosphate from other industries, such as food preservatives, anticorrosion agents, cosmetics, fungicides, ceramics, water treatment, and metallurgy, all providing costlier products, and (3) sources of high quality phosphates are rapidly depleted and expected to be exhausted in less than 100 years (Middleton 2003). Annual consumption of phosphate rock has approached 150 million metric tons; about 95 % of this production is used in the fertilizer industry (Dorozhkin 2011).

Inorganic P occurs in soil, mostly in insoluble mineral complexes, some of them appearing after frequent application of chemical fertilizers. These insoluble, precipitated forms cannot be absorbed by plants (Rengel and Marschner 2005). Organic matter is also an important reservoir of immobilized P that accounts for 20–80 % of P in soils (Richardson 1994). Only 0.1 % of the total P exists in a soluble form available for plant uptake (Zou et al. 1992).

The original source of most soil and plant P is apatite (Ahn 1993). Under natural conditions in soil and in seawater, P rapidly precipitates as forms of sparingly soluble complexes of different kinds of phosphates. The most common ones in acid agricultural soils are variscite ($\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$), followed by strengite ($\text{FePO}_4 \cdot 2\text{H}_2\text{O}$). Both are very stable minerals (Richardson 2001). In alkaline soils having abundant calcium, there is no detectable orthophosphate (PO_4^{3-} , Pi) (Goldstein et al. 1999). The most stable minerals are calcium phosphates, which are, in order of decreasing solubilities, dicalcium phosphate dihydrate (brushite) $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ > anhydrous dicalcium phosphate (monetite) CaHPO_4 > octacalcium phosphate $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ > tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2$ > hydroxyapatite $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ > fluorapatite $\text{Ca}_5(\text{PO}_4)_3\text{F}$ (Haynes 1982; Wang and Nancollas 2008; Dorozhkin 2011). Stable forms for organic P are phytate (dodecasodium inositol hexaphosphate, $\text{Na}_{12}\text{C}_6\text{H}_6\text{P}_6\text{O}_{24}$) and for rock phosphate (sedimentary rock containing phosphate minerals, phosphorite) are apatite, fluorapatite, and hydroxyapatite (Hoffland 1992). The low solubility of these stable minerals makes them unavailable for plants (using soluble Pi for growth). This results in frequent shortage of Pi for plant nutrition, even though the soil may contain a high level of total P (Merbach et al. 2010).

For the above reasons, agricultural research focused on the following: (1) low-grade rock phosphate (9–11 % P_2O_5 or less) as a source of fertilizer in the future because low-grade ore is available worldwide in large quantities (Rajan et al. 1996; Bationo et al. 1997) and (2) other sources of phosphate, such as struvite derived from wastewater treatment (de-Bashan and Bashan 2004). This trend especially happens in developing countries where P availability for crops is more acute. In rock phosphate used as fertilizer, where its insoluble phosphate is almost unavailable for plant growth, phosphate-solubilizing microorganisms are used to

transform the insoluble phosphate into available soluble phosphate. This is the most common and logical approach (Reyes et al. 2001; Whitelaw 1999; Richardson 2001; Rodriguez et al. 2006).

Phosphate-solubilizing bacteria

The history and first experimental findings pointing to the important role of soil microorganisms in solubilizing phosphate minerals, making Pi available to plants, is at least century-old knowledge, as outlined in the landmark paper by Gerretsen (1948) and later by Goldstein (Goldstein 2007; Goldstein and Krishnaraj 2007). For example, one of the earliest reports date back to 1908 (Sackett et al. 1908; cited from Greaves 1922).

In recent years, many publications presented a very large number of new phosphate-solubilizing bacteria (PSB). Some involved isolation of thousands of new strains (for example, Mehta and Nautiyal 2001; Peix et al. 2003, 2004; Chung et al. 2005; Chen et al. 2006; Reyes et al. 2006; Gulati et al. 2008; Jorquera et al. 2008) or were offered directly by inoculant companies without any publication record. High percentage of those were preliminary studies, conducted solely in vitro, and lacking plant and field application. Many of these studies assumed that in vitro P solubilization capacity will translate into available P for plant nutrition under usual growing conditions in soil. Some optimistic reports notwithstanding (Kumar and Narula 1999; Harris et al. 2006), this is by far not the case (Rengel and Marschner 2005). The literature abounds with microbiological reports on successful in vitro solubilization of P that could not be repeated under field conditions (Gyaneshwar et al. 2002; Rengel and Marschner 2005).

To isolate a suitable potential PSB, a defined selective medium lacking available sources of soluble P, apart from insoluble P, is the right strategy. Theoretically, this will ensure that any isolate growing in this medium has phosphate-solubilizing ability. Such a growth medium was initially proposed in 1948 by Pikovskaya (1948). This medium contains, as its sole selection factor, tricalcium phosphate [TCP, $\text{Ca}_3(\text{PO}_4)_2$] with a low-medium rate of solubilization (Haynes 1982). With time, TCP became the selection factor for isolating new PSB or for demonstrating phosphate-solubilizing capacity in numerous studies (Kumar and Narula 1999; Rodriguez and Fraga 1999; Katiyar and Goel 2003; Chen et al. 2006; Rajkumar et al. 2006; Son et al. 2006; Ahmad et al. 2008; Jorquera et al. 2008; Oliveira et al. 2009; Park et al. 2010; Liu et al. 2011; Table 1). Later, the medium was modified, but the modification still contains TCP as a selection factor (India's National Botanical Research Institute (NBRIP) phosphate

Table 1 Literature samples of potential phosphate-solubilizing bacteria, some also tested as plant growth-promoting bacteria

Type of insoluble phosphate	Plant species (isolation)	Total number of PSB isolates	Number of proven PSB-PGPB	Plant species (testing)	Reference
TCP	Undefined plant species and soils	2015	NE	NE	Mehta and Nautiyal (2001)
TCP	Undefined native vegetation	36	NE	NE	Chen et al. (2006)
TCP	Onion, pepper, sesame, rice	Several hundred	NE	NE	Chung et al. (2005)
TCP	Sea buckthorn	216	NE	NE	Gulati et al. (2008)
TCP	Ginseng	1	NE	NE	Park et al. (2010)
TCP, Na-phytate (tested separately)	Perennial ryegrass; white clover, wheat, oat, yellow lupin	1500	NE	NE	Jorquera et al. (2008)
TCP	Several crops from northern India	66	NE	NE	Ahmad et al. (2008)
TCP	Soils	446	NE	NE	Alikhani et al. (2006)
TCP	Mesquite	857	NE	NE	Johri et al. (1999)
TCP	Soybean	1	NE	NE	Son et al. (2006)
TCP	Halophytic native plants	70	NE	NE	Xiang et al. (2011)
TCP	Poplar	74	NE	NE	Liu et al. (2011)
TCP	Soil	33	NE	NE	Viruel et al. (2011)
Hydroxyapatite	Five plant species colonizing mine tailings	15	NE	NE	Reyes et al. (2006)
Hydroxyapatite	Phosphate mine rocks	52	NE	NE	Ben Farhat et al. (2009)
Rock phosphate	Several legumes	30	NE	NE	Bardiya and Gaur (1974)
Rock phosphate	Unknown	2	NE	NE	Xiao et al. (2011)
TCP	Yerba mate	518	1	Common bean	Collavino et al. (2010)
TCP	Soybean	13	None	Soybean	Fernández et al. (2007)
TCP	Peanut	110	1	Peanut	Taurian et al. (2010)
TCP	Lotus	50	3	Lotus	Castagno et al. (2011)
TCP	Walnut	34	2	Walnut	Yu et al. (2011)
TCP	Garlic	1	1	Wheat	Selvakumar et al. (2009)
TCP	Several crops	81	2	cowpea	Linu et al. (2009)
TCP	Field soil	21	1	Wheat	Ogut et al. (2010)
TCP	Black mangrove			<i>Salicornia</i>	Bashan et al. (2000), Vazquez et al. (2000)
TCP	Mangrove	129	1	Mangrove	El-Tarabily and Youssef (2010)
TCP	Wheat	164	5	Wheat	Kumar and Narula (1999)
TCP	Salt-affected soil	23	5	Sorghum	Srinivasan et al. (2012)
TCP	Stevia	12	5	Stevia	Mamta Rahi et al. (2010)
TCP	Three weeds	3	3	Maize	Naz and Bano (2010)
Dicalcium phosphate or phytic acid	Soil	□300	5	Maize, lettuce	Chabot et al. (1996)
Dicalcium phosphate	Various culture collections	143	2	Radish (<i>Raphanus sativus</i>)	Antoun et al. (1998)
Phytate	White lupin	>300	16	White lupin	Unno et al. (2005)
Phytate	Soil	3	3	Maize	Idriss et al. (2002)
Phytate	Soil and chicken manure	10	NE	NE	Hill et al. (2007)
Ca ₁₀ (OH) ₂ (PO ₄) ₆ , AlPO ₄ , FePO ₄ ·2H ₂ O	Cardon cactus	20 in rhizoplane, 26 endophytes	4 in rhizoplane, 6 endophytes	Cardon cactus	Puente et al. (2004a, b, 2009a, b)
Ca ₁₀ (OH) ₂ (PO ₄) ₆	<i>Mammillaria</i> (cactus)	10	4	<i>Mammillaria</i>	Lopez et al. (2011)
Rock phosphate	Wheat, maize	35	6	Wheat	Baig et al. (2012)
Ca ₃ (PO ₄) ₂ CaF ₂	Undefined	4	4	Pulse	Iqbal et al. (2010)
Zn ₃ (PO ₄) ₂ ·4H ₂ O	Several crops	9	9	Canola	de Freitas et al. (1997)

Table 1 (continued)

Type of insoluble phosphate	Plant species (isolation)	Total number of PSB isolates	Number of proven PSB-PGPB	Plant species (testing)	Reference
Rock phosphate or CaHPO ₄					
AlPO ₄	Forest soils	4 (including 3 fungi)	1	Garden cress	Illmer et al. (1995)
Rock phosphate		1	1	Onion	Vassilev et al. (1997)
Rock phosphate	Wheat	8 (including 2 fungi)	1 (including 2 fungi)	Wheat	Babana and Antoun (2005, 2006)
Rock phosphate	Rice	16	3	Rice	Rajapaksha et al. (2011)
Rock phosphate	Compost and macrofauna	5	2	Maize	Hameeda et al. (2008)

Black mangrove (*Avicennia germinans*), canola (*Brassica napus*), cardon cactus (*Pachycereus pringlei*), common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), garden cress (*Lepidium sativum*), garlic (*Allium sativum*), ginseng (*Panax ginseng*), lettuce (*Lactuca sativa*), lotus (*Lotus tenuis*), *Mammillaria* (*Mammillaria fraileana*), mangrove (*Avicennia marina*), Mesquite (*Prosopis juliflora*), oat (*Avena sativa*), onion (*Allium fistulosum*), peanut (*Arachis hypogaea*), pepper (*Capsicum annuum*), perennial ryegrass (*Lolium perenne*), poplar (*Populus* sp.), pulse (*Vigna radiata*), radish (*Raphanus sativus*), rice (*Oryza sativa*), *Salicornia* (*Salicornia Bigelovii*), sea buckthorn (*Hippophae rhamnoides*), sesame (*Sesamum indicum*), sorghum (*Sorghum bicolor*), soybean (*Glycine max*), stevia (*Stevia rebaudiana*), walnut (*Juglans siggillata*), wheat (*Triticum aestivum*), white clover (*Trifolium repens*), white lupin (*L. albus*), yellow lupin (*Lupinus luteus*), yerba mate (*Ilex paraguariensis*)

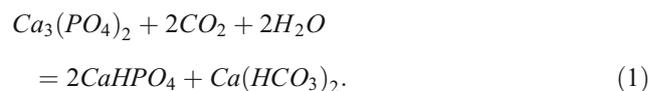
NE not evaluated on plants

growth medium; Nautiyal 1999). This medium was proposed as a suitable and appropriate medium for the task of isolating and testing PSB and is widely used. In addition to TCP, other P compounds are used for isolating and testing PSB, such as rock phosphate (hydroxyapatite; Reyes et al. 2006), dicalcium phosphate (Chabot et al. 1996; Antoun et al. 1998; Peix et al. 2003, 2004), and phytate (Chabot et al. 1996; Unno et al. 2005), but used to a far lesser extent.

It appears that TCP, although a potentially insoluble P, is not hard to dissolve, compared with other harder-to-dissolve phosphates. Pérez et al. (2007) isolated 130 strains capable of solubilizing TCP from thousands of colonies developed on NBRIP medium. None of those showed solubilizing activities of iron phosphate (FePO₄) or aluminum phosphate (AlPO₄), which renders their usefulness largely impractical for acidic soils. From TCP plates, Park et al. (2010) isolated an efficient PSB that also solubilized FePO₄ and AlPO₄. Yet it solubilized TCP 10 to 50 times better. Similar results in solubilizing were obtained by Song et al. (2008) and Chang and Yang (2009), using additional hydroxyapatite and rock phosphate as sources of P. When four kinds of P (TCP, AlPO₄, phytate, and soybean lecithin) were compared for isolating PSB, the biggest number of strains was obtained from TCP plates and they solubilized best this P material (Oliveira et al. 2009). Comparison of several PSB *Azotobacter chroococcum* strains to solubilize TCP and Mussoorie (India) rock phosphate showed that the latter is hardly solubilized (Kumar and Narula 1999). Even cloned *Escherichia coli*, having no relation to plant nutrition, is capable of solubilizing TCP (Goldstein and Liu 1987; Kim et al. 1997, 1998).

By this test, even *E. coli* would be considered a PSB. It appears that numerous bacterial strains that produce organic acid from metabolism of sugars, especially metabolizing glucose to strong gluconic and 2-ketogluconic acids, are capable of dissolving TCP (Mehta and Nautiyal 2001; Chen et al. 2006; Goldstein 2007; Goldstein and Krishnaraj 2007; Trivedi and Sa 2008). Therefore, all these bacteria are presumably PSB, which would be an absurd conclusion.

Finally, as mentioned in the report by Greaves (1922), TCP is several times more soluble in CO₂-saturated water than in pure water, resulting in its gradual transformation to more soluble Ca salts. This happens according to the following general equation:



In summary, any TCP-based selective medium that isolated hundreds or thousands of potential PSB strains from a soil is not selective enough for the task of selection.

PSB and plant growth-promoting bacteria

With time, evidence accumulated in the literature reporting potential PSB candidates from in vitro studies that very few were also plant growth-promoting bacteria (PGPB). PSB and plant growth-promoting bacteria (PSB-PGPB) are the end-product microorganisms to be used in agriculture as inoculants and are the declared main final goal of all the PSB studies. For example,

Collavino et al. (2010) found that there is no correlation between potential PSB isolated on TCP and their ability to promote plant growth. Fernández et al. (2007) tested 13 efficient TCP-solubilizing strains from soil and found that none promoted plant growth nor improved plant P nutrition. Taurian et al. (2010) tested 110 potential PSB on TCP and found only one that could promote peanut growth. El-Tarabily and Youssef (2010) isolated 129 TCP-solubilizing bacteria but only one was effective as a PSB-PGPB for mangrove seedlings. An efficient PSB-PGPB for maize exhibited much higher solubilization of TCP than for three types of rock phosphate (Gulati et al. 2010). Yu et al. (2011) isolated a large number of PSB on TCP medium yet could find only two PSB-PGPB; they propose that more insoluble P materials should be selected to isolate potential PSB-PGPB. At the same time, studies that used rock phosphate or other hard-to-dissolve phosphates were more successful in isolating PSB-PGPB, but not necessarily with phosphate solubilization as the main mechanism (de Freitas et al. 1997; Puente et al. 2004a, b, 2009a, b; Lopez et al. 2011, 2012; Baig et al. 2012). In summary, regardless of the promise of PSB, the multitude of strains isolated, and the several microbial inoculants in the marketplace, successful application in the field is still very low.

Analysis of the literature

Here, we extracted data from common scientific literature on PSB, their origin, selection factor for their isolation, and their performance as PSB-PGPB, and considered the potential chemical solubilization pathways for several hard-to-dissolve phosphates by potential PSB and those available in the rhizosphere of plants. This was done to reach an initial conclusion about which insoluble phosphate(s) is most useful to serve for isolating and testing potential PSB-PGPB.

This approach was chosen because, among the several mechanisms responsible for phosphate solubilization in soil (Illmer and Schinner 1995), production of organic acids by plant roots and their associated microbes (bacteria and fungi) plays *the* major role. In many bacteria, the direct oxidation pathway (also called non-phosphorylating oxidation) leads to the production of gluconic and 2-ketogluconic acids directly into the periplasmic space, where their protons are efficiently released into the extracellular medium, thus lowering its pH. These strong organic acids, having extremely low K_a values, $\log K_a = 3.4$ and $\log K_a = -2.6$, respectively (Goldstein and Krishnaraj 2007), can dissolve difficult-to-dissolve calcium phosphates, such as hydroxyapatite and rock phosphate ore, such as fluorapatite. The

bacteria exhibiting nonphosphorylating oxidation were designated as having the MPS⁺ (mineral phosphate-solubilizing) phenotype (Goldstein 1995). Goldstein (2007) proposes that conservation of the direct oxidation pathway in rhizobacteria may, at least in part, result from the mutualistic advantage provided by the MPS trait.

Acids are well known to dissolve rock phosphate (Kpombekou and Tabatabai 1994). The most common organic acids produced by potential PSB are not only gluconic, 2-ketogluconic, citric, oxalic, succinic, propionic, and acetic but also isovaleric, heptanoic, caproic, formic, *n*-butyric, oxalic, and methylmalonic were detected (Chen et al. 2006; Puente et al. 2009a). In alfalfa, pea, lupin, sorghum, maize, wheat, and barley, malate and citrate are the common organic acids. These organic acids appear to be the primary components released by roots with P deficiency (Jones and Darrah 1994; Jones 1998). White lupin (*Lupinus albus*) exudates also contain fumaric, *cis*-aconitic, and *trans*-aconitic acids. Chickpea (garbanzo, Bengal gram; *Cicer arietinum*) was the only species that contained malonic acid, in addition to malic, fumaric, and *cis*- and *trans*-aconitic acids (Cawthray 2003). In general, all these acids chelate cations (mainly Ca²⁺, also Fe³⁺ and Al³⁺) bound to phosphate through their hydroxyl and carboxyl groups or solubilize them by the liberation of protons, thereby converting insoluble P into soluble forms that are available for plant nutrition (Kpombekou and Tabatabai 1994).

General considerations on dissolution of phosphate minerals

To clarify the theory of rock phosphate dissolution, first, one needs to consider a simple process of dissolution of dissociating salts (including metal phosphates, the subject of this assay) in pure water or in slightly acidic or alkaline medium and disregarding any hydrolysis or complexing (see below). Solubility is defined as the maximal concentration reached under specified conditions in the binary system solid phase–solution without supersaturation. It is a thermodynamic value, which could ideally be reached for a well-crystallized stable phase in equilibrium with the solution (also see below). It is determined by the value of the solubility product for this solid phase. The solubility product is a product of the concentrations (ideally = *in the ideal case* activities, which can be substituted by concentrations when they are very low) of all the ions (formed upon dissociation of the salt) in the powers of their stoichiometric coefficients. As an example, for a salt M_xA_y (where M is a metal, A is an anion, disregarding the charges; *x* and *y* are the stoichiometric coefficients in the chemical formula), the solubility product

constant (K_S) for the dissociation equilibrium (2) is calculated as shown in Eq. (3).



$$K_S = [M]^x \times [A]^y. \quad (3)$$

The K_S value is a constant for a stable, solid, well-crystallized phase of definite composition and can generally be used for calculating the aqueous solubility of a solid or any of the ions (M or A), if the other's concentration in equilibrium is known. For orthophosphates, where there is also an equilibrium in solution between the protonated and nonprotonated ions ($H_2PO_4^-$, HPO_4^{2-} , and PO_4^{3-}), which depends on the pH, the dependence of solubility on pH can also be generally calculated (Wang and Nancollas 2008; Dorozhkin 2011).

In principle, K_S values may be found in the literature for various phosphate minerals for Ca phosphates (Wang and Nancollas 2008; Dorozhkin 2009, 2011) and Al and Fe^{III} phosphates (Stumm and Morgan 1996; Jiang and Graham 1998). For $AlPO_4 \cdot 2H_2O$ and $FePO_4 \cdot 2H_2O$ (Bache 1963), different K_S values (stated to be valid only at low pH) were given which are based on the dissociation scheme similar to Eq. (2), which however, additionally includes hydration water molecules and, consequently, water activity in the corresponding expression is similar to Eq. (3) for K_S . This is not typical for aqueous solutions of low concentrations, where water activity can be assumed to be constant, and can be confused with calculated K_S values that exclude water (Wang and Nancollas 2008). This could only complicate relevant calculations.

As mentioned above, K_S , as a thermodynamic value, refers to the equilibrium conditions of the chemically uniform (homogeneous) and well-crystallized stable, solid phase only. In reality, however, there are four reasons that often make an ideal K_S value virtually useless:

- The solubility product strongly depends on the crystalline state (and on the thermodynamic stability) of a mineral (see below). Often, there is no reliable information under which conditions and for which solid phase the solubility product was measured for calculating K_S .
- In a medium where the ionic strength (i.e., background concentrations of other inert ions) is not very low (as what happens in real solutions), the activity coefficients (Wang and Nancollas 2008) may significantly differ from unity (=1) and render the values of activities and concentrations no longer equivalent (activity = concentration multiplied by activity coefficient). If the latter is not close to unity, activity becomes different from concentration (i.e., they become nonequivalent).

Thus, the literature data on K_S values determined at low ionic strengths become inapplicable. For example, the value of pK_S ($= -\log_{10} K_S$) for $AlPO_4 \cdot 2H_2O$ at zero ionic strength (i.e., almost pure water) was reported to be $pK_S=21$, i.e., $K_S=1.0 \times 10^{-21} M^2$ (Stumm and Morgan 1996; Jiang and Graham 1998). However, at ionic strength 0.15, it was reported to be $pK_S=18.3$ (Duffield et al. 1991; Martin 1997), i.e., $K_S=5.0 \times 10^{-19} M^2$. Thus, already at such a relatively small ionic strength, which is equivalent to that of the physiological solution (0.88 % NaCl), the K_S is 500 times higher than in pure water, and, consequently, the solubility of $AlPO_4$ is increased by $(500)^{1/2}$ (~22 times).

- Even a small impurity (that is, a small percent in the whole substance) of a less-crystalline (i.e., more amorphous) phase may give an apparent result of a higher dissolution level and/or rate (see below about the dissolution rate). Thus, the solubilization test would be wrong. It would give a higher solubilization rate than the real rate for the main substance and a higher solubility caused solely by the less-crystalline substance and more soluble impurity.
- In real systems, when the phosphate mineral can be dissolved by an acid, the rate of dissolution/solubilization, identified as the amount of mineral dissolved per unit of time, may vary greatly, from a very high rate (very fast dissolution) to a very low rate (very slow dissolution). This solubilization rate refers not to the thermodynamics of dissolution (i.e., equilibrium state), but to the chemical kinetics. The kinetics of solubilization (dissolution) depends on a number of other parameters, one of the most important of which is the specific surface area of the mineral. This is because the solubilization reaction is a heterogeneous process (i.e., occurring between a liquid phase and a solid phase, not within a single phase) and proceeds exclusively at the surface of a mineral. In other words, its surface-exposed moieties or structural units, which are in contact with the solution, react with the ions adsorbing onto them from the solution. Therefore, the greater the surface area of a mineral sample (note that smaller crystallites and higher porosity increase the surface area per unit of mass), the faster is the rate of solubilization.

To conclude, calculations of solubilities using solubility product data (K_S constants from the literature) as the data related to ideal *equilibrium states* in solutions would provide no information regarding the rate of true real-life solubilization. In particular, the K_S values of phosphate minerals, which might be considered as a first approximation to their solubility, in view of the aforementioned, do not give any reliable information. Additionally, K_S values do not

take into account the complexing ability of an organic acid's anion (see below).

Any reliable information on the ability of organic acids to dissolve poorly insoluble phosphates, including the dissolution rate, could be obtained exclusively in model experiments that should include different organic acids (pure substances) and different reference phosphate minerals, each of a definite formula (composition), chemical state (crystallinity, stability, phase homogeneity), and specific surface area. Such experimental data, obtained with the same mineral for different acids and with each acid reacting with different phosphate minerals, could be comparable enough to make valid conclusions about which acids are strong solubilizers and which mineral is more or less easy to solubilize. A database of such results could be used to determine the ability of a potential PSB to solubilize different phosphate minerals in relation to the spectrum of organic acids produced by the bacteria.

Crystallinity and solubility—general chemical considerations

While assessing and comparing the biological availability of phosphate minerals, the following important comment by Gillis et al. (1962) should be noted: “Some confusion exists in the literature because investigators not always recognized that many phosphate compounds may exist in several states of hydration and crystal modification.” This is of paramount importance with regard to biological phosphate solubilization discussed here. Different crystal modifications including not only different hydration states but also the level of crystallinity of *the same* substance usually have different water solubilities and different dissolution rates either in water or in acidified solutions. More amorphous phases usually have higher solubilities. This is directly related to their thermodynamic state.

As described above, the solubility (as a thermodynamic value) of a solid substance represents its concentration in solution in equilibrium with the stable solid phase. From thermodynamic considerations, the solubility tends to decrease with increasing crystallinity; therefore, most highly crystalline material has the lowest solubility. Also, more amorphous phases are commonly more rapidly dissolved. In particular, this is because of their higher specific surface area, as compared to that of a strongly crystallized phase, which favors dissolution kinetics. Therefore, for poorly soluble materials (such as phosphates), even a small admixture of an amorphous phase could dramatically affect the results of investigations on their solubility or dissolution rate and, as a consequence, misrepresent the corresponding bioavailability tests. For example, it is common that in a stable solid

crystalline, especially in a natural mineral, there is a small impurity (often a few percent or even lower) of a more amorphous phase of the same substance. While dissolving, this impurity being preferentially and more rapidly dissolved may give an apparent, but not real, higher solubility. It has to be noted that such a small impurity may well be not easily detectable, being totally or almost invisible for X-ray diffraction or spectroscopic techniques. Therefore, any differences in PSB tests performed in different laboratories with the same mineral, which, in fact might differ by such impurities, could be misinterpreted as real differences between the PSB properties.

The aforementioned consideration clearly implies that, to test and compare the bioavailability of phosphate minerals for potential PSB, some stable and uniform (structurally and chemically homogeneous), well-crystallized phases should only be chosen as standards. They should have a number of defined characteristics such as spectra, thermograms, and X-ray diffraction patterns which must be checked or otherwise ensured to be valid before performing a test with PSB. This is particularly important, as for many metal phosphate minerals, different crystal modifications are known, including calcium orthophosphates (Wang and Nancollas 2008; Dorozhkin 2009, 2011). In addition, if such a test phosphate material is to be prepared in a laboratory to perform PSB tests, there should be some treatment steps developed and commonly recommended to obtain a stable and structurally homogeneous mineral phase to be used by different researchers in laboratories throughout the world.

Regarding Ca orthophosphates (including TCP because it contains PO_4^{3-}), and specifically TCP, the most stable and the least soluble modification of the latter is $\beta\text{-Ca}_3(\text{PO}_4)_2$ (with $\text{p}K_S = -\log_{10}K_S = 28.9$ and solubility of $\sim 0.5 \text{ mg L}^{-1}$ at 25°C), while there is its higher temperature polymorph $\alpha\text{-Ca}_3(\text{PO}_4)_2$. Both polymorphs are stable at room temperature in the absence of humidity (Dorozhkin 2011). It has to be mentioned that neither polymorph can be precipitated from aqueous solutions (Wang and Nancollas 2008; Dorozhkin 2009, 2011). Therefore, for any PSB tests, $\beta\text{-TCP}$ has to be either taken as a mineral (which might imply the presence of different impurities for minerals of different origin), which could affect the PSB tests or prepared using a high-temperature synthesis in the laboratory (which could easily be standardized).

Nevertheless, it should be noted that, among Ca-phosphate minerals, the most stable and least soluble is thought to be fluorapatite $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$ ($\text{p}K_S = 120$), with solubility of $\sim 0.2 \text{ mg L}^{-1}$, stable within pH 7–12 (at 25°C), while hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ($\text{p}K_S = 117$) is close with the solubility of $\sim 0.3 \text{ mg L}^{-1}$, stable within pH 9.5–12 (Dorozhkin 2011).

Types of microbially driven mineral phosphate dissolution processes

The processes resulting in mineral phosphate solubilization, in particular, those driven by soil microorganisms, in principle involve several chemically different reactions (Table 2):

1. The most straightforward process is *simple acidification* of the medium as a result of proton release, for example, as the H⁺ antiport in the course of bacterial ammonium (NH₄⁺) assimilation, by means of which the cell maintains a neutral charge, or production of inorganic acids that do not form strong complexes with Ca, Al, or Fe^{III} and easily release protons upon dissociation (Illmer and Schinner 1995; Illmer et al. 1995; Rodriguez and Fraga 1999; Whitelaw 1999). The reaction scheme is as follows, as in the case of TCP, resulting in the formation of more soluble phosphates:



While the solubility of calcium phosphate increases exponentially with decreasing pH (Merbach et al. 2009), the behavior of AlPO₄ and FePO₄ is different (Fig. 1). The solubility of ferric phosphate decreases with lower pH down to 4.5–3.5, and aluminum phosphate has the lowest solubility within pH 5.5–4.5, for example, at pH 3.5 it is comparable with that at pH 7. Hence, acidification of the medium per se cannot account for phosphate mobilization in bacterial cultures in the cases of aluminum or ferric phosphates (Fankem et al. 2008; Merbach et al. 2009).

In accordance with the literature (see Fig. 1), aluminum phosphate is considerably more soluble than the corresponding ferric salt (Brosheer et al. 1954; Ishio et al. 1986; Fankem et al. 2008). Similarly, according to He et al. (2006), in 0.322 mM KH₂PO₄ solution, the addition of FeCl₃ or AlCl₃ (3.22 mM) in 100 mM acetate buffer (at pH 5.0, 22 °C) after 20 h precipitates 60 % or only 5 % of soluble inorganic phosphate, respectively.

Table 2 Main general types of microbially driven processes resulting in mineral phosphate dissolution

Type of process	Main cause/entity of mineral dissolution	Main reaction leading to mineral dissolution	Applicability to phosphate minerals
1. Acidification of the medium	Release of protons (H ⁺) or production of easily dissociating inorganic acids	Lowering the pH of the medium, formation of more soluble hydrophosphates	Ca phosphates
2. Metal complexing	Release of organic acids or complexing (chelating) agents	Formation of metal complexes (including chelates in cases of di-, tricarboxylic, or hydroxocarboxylic acids) ^a	Ca, Al, Fe phosphates
3. Metal reduction	Redox activity of bacteria or their exudates (secondary metabolites)	Reduction of the metal with variable oxidation states (bound to phosphate) to a lower oxidation state (resulting in a more soluble phosphate)	Fe ^{III} phosphate ^b
4. Enzymatically driven phosphate dissolution	Extracellular release of specific enzymes (phosphatases)	Enzymatic hydrolysis of poorly soluble organic phosphate esters releasing inorganic phosphate	Various organic phosphate esters (phytate, phospholipids)
5. Indirect phosphate dissolution	Microbial stimulation of organic acid exudation by plants	The same as for type 2 (metal complexation) but related to plant–microbe interactions ^c	Ca, Al, Fe phosphates

^a Such reactions can result in the formation of poorly soluble metal complexes. In that case, inorganic phosphate would be solubilized not because of the dissolution of the initial mineral but rather because of the “ligand exchange” process, where the complexing ligand substitutes for phosphate releasing the latter (“substitutional phosphate solubilization”). Importantly, such reactions can give no “phosphate solubilization halo” in PSB agar tests (see below) which could thus be misinterpreted as the absence of phosphate solubilization. In such cases, parallel tests in a liquid medium (with an analysis for liberated phosphate) should be performed

^b Can be applicable to other redox-active metals such as Mn^{3+/4+}-containing phosphate minerals, as well as to phosphates chemisorbed to oxyhydroxides of redox-active metals (phosphate-containing ferric oxides/oxyhydroxides)

^c May in principle be related to types 1 or 3 (in case of microbial stimulation of plant exudation of protons or metal-reducing substances, respectively) or to type 4 (in case of microbial stimulation of plant exudation of relevant enzymes)

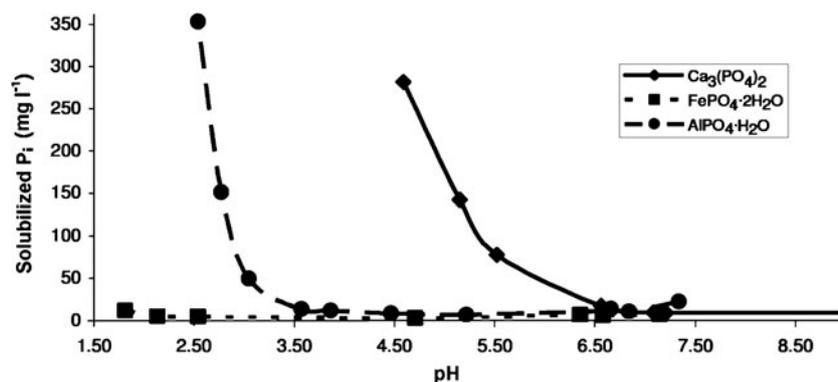
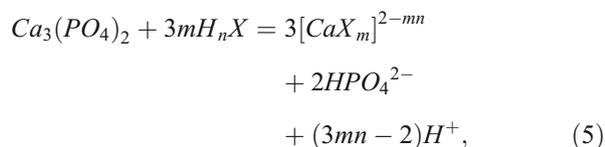


Fig. 1 Solubilization of phosphate from Ca, Al, and Fe^{III} phosphates as a function of pH (adjusted using HCl or NaOH, measured after phosphate extraction). The extraction was made by mixing 100 mg of a solid phosphate in 30 mL solution under stirring for 90 min at 150 rpm.

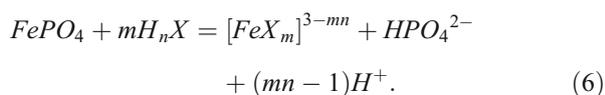
At the end of the incubation period, the solution was centrifuged at 6,000×g, its pH was measured, and the phosphate in solution was determined (information adapted from Fankem et al. (2008))

2. *The formation of a metal complex in solution*, where the metal ion is coordinated by an anion. Specifically, metal chelation, in the case of a chelating ligand or anion that forms two or more bonds with the metal forming a ring structure, transforms an insoluble phosphate mineral into the metal complex and releases phosphate anions. The reaction equation is as follows, as in the case of TCP, and a complexing acid H_nX:



where *m* is the stoichiometric coefficient in the calcium complex formed, *n* is the index equal to the absolute value of the charge of the complexing anion (Xⁿ⁻).

The excessive protons in Eq. (5) might be involved in parallel processes of phosphate solubilization, such as in Eq. (4), or get bound to other anions, depending on the pH of the medium. A similar process could be applicable to the less-soluble phosphates of Fe^{III} or Al, according to the following equation:



Note that Al and Fe^{III} complexing (in particular, chelation) seems to be the main mechanism for the microbially driven dissolution of aluminum and ferric phosphate minerals. It is related to the well-known phenomenon of synthesis and release of a range of organic acids by many bacteria, in

particular, induced by phosphate deficiency (Chen et al. 2006; Puente et al. 2009a). Phosphate may also be solubilized, not only from rock phosphate minerals but, via ligand exchange involving organic acid anions, from other minerals, such as oxides containing chemisorbed phosphate anions (Arcand and Schneider 2006).

Halo formation on solid agar, produced by developing bacterial colonies, has served as a universal indicator for phosphate solubilization by PSB for over half a century. As indicated in Table 2 (footnote a), special care should be taken, as the formation of insoluble Ca (and sometimes also Al or Fe^{III}) complexes could solubilize phosphate but may give no solubilization halo on the agar plates. As a solution for this difficulty, such PSB test plates should be complemented by liquid culture tests or genetic characterization of potential PSB (Merbach et al. 2009). For Ca phosphate minerals, such poorly soluble complexes, preventing the formation of a solubilization halo, can be formed with oxalate, tartrate (Arcand and Schneider 2006), phytate (Evans and Pierce 1981; Grynspar and Cheryan 1983; for poorly soluble phytate complexes of Al and Fe^{III} (He et al. 2006)), and even with citrate (Merbach et al. 2009) anions, while citrate readily dissolves AlPO₄ (Martin 1997). Lobartini et al. (1998) mention that humic and, to a lesser extent, fulvic acids were useful chelating agents for Al³⁺ and Fe³⁺ and were effective in dissolving AlPO₄ and FePO₄. However, humic soil substances may contain organic matter–metal (Al³⁺ and/or Fe³⁺) phosphate complexes (He et al. 2006) that contribute to phosphate solubilization via ligand exchange, primarily with metal-complexing or chelating organic acid anions.

Formation of soluble and insoluble Ca, Al, or Fe^{III} complexes with organic acid anions would dramatically reduce the concentrations of free (hydrated) ions in the medium. Thus, by lowering the solution saturation point, this would result in shifting the mineral dissolution equilibria and facilitating the dissolution processes (Welch et al. 2002).

- For the redox-active cation (in the case of Fe^{III} phosphate only, FePO₄), a preliminary *metal reduction step*, either directly (for Fe³⁺-reducing bacteria) or indirectly microbially driven (such as via release of reductive secondary metabolites), may lead to the formation of Fe^{II}. It has long been known that poorly soluble ferrous (Fe²⁺) salts commonly are still noticeably more soluble than the corresponding ferric (Fe³⁺) salts.

In particular, Fe^{II} phosphate was reported to be moderately available to plants (Gerretsen 1948). Special studies (Ghassemi and Recht 1971) showed that Fe^{II} orthophosphate (Fe₃(PO₄)₂·8H₂O, vivianite) has a sharp minimum in solubility at pH 8.0, while the solubility steeply rises both at higher and lower pH.

This type of phosphate solubilization can be applicable to a wide range of other redox-active metals, such as Mn^{2+/3+/4+}-containing phosphate minerals (Schwab 1989), as well as phosphates chemisorbed to oxyhydroxides of many other redox-active metals, such as phosphate-containing ferric oxides/oxyhydroxides.

- Enzymatically driven dissolution of phosphate may be an essential process in organic phosphorus-rich soils, involving phosphatase-driven hydrolysis of poorly soluble organic phosphate esters, which release inorganic phosphate (Rodriguez et al. 2006; Nannipieri et al. 2011). However, in the presence of Al³⁺ and Fe³⁺, enzymatic release of phosphate from phytate by incubation with a fungal phytase was affected. This resulted from formation of insoluble Al³⁺ or Fe³⁺ phytate complexes, rather than precipitation of soluble orthophosphate after its enzymatically driven release from phytate (He et al. 2006).
- Indirect solubilization, such as microbial stimulation of exudation of organic acids by plants (Arcand and Schneider 2006), by its mechanism is close to type 2 solubilization (listed above). However, it is directly related to plant–microbe interactions in the rhizosphere and thus is relevant to rock phosphate solubilization in extensively planted agriculture soils (see also Table 2).

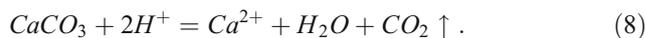
Sometimes, in the PSB-related literature, contradictory results are found. For example, when inoculation of plants with phosphate-solubilizing bacteria occurs, increased uptake of P from soil (Kucey et al. 1989), the phosphate-solubilizing activity of the strains studied by Belimov et al. (2002) is unimportant in P uptake by inoculated plants.

The influence of coexisting carbonates on mineral phosphate solubilization

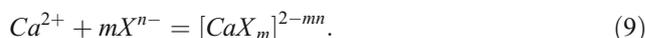
If there are suitable conditions for saturation of an aqueous medium with carbon dioxide (CO₂), it might facilitate dissolution of calcium phosphate minerals (see general Eq. (1)), since it can lower the pH to 3.8, which is the pH of a saturated CO₂ solution at room temperature (Gerretsen 1948). This results from the equilibrium with partial formation of a very weak carbonic acid that weakly dissociates, according to Eq. (7):



Nevertheless, in the presence of coexisting mineral carbonates, microbially driven mineral phosphate solubilization may be significantly retarded. This particularly relates to the phosphate solubilization mechanisms involving acidification of the medium, for example, antiport of protons (H⁺) in the course of NH₄⁺ assimilation or release of organic/inorganic acids. In this case, a parallel reaction involves the carbonate-containing phase, such as for calcium carbonate:



In this case, as what follows from the chemistry of heterogeneous processes, formation of the gaseous phase (CO₂) and its removal from the reaction medium (volatilization) shifts the equilibrium toward carbonate dissolution, rather than possible parallel solubilization of the coexisting poorly soluble mineral phosphate. In this case, the consumption of protons in Eq. (8) prevents acidification of the medium and, correspondingly, retards mineral phosphate solubilization. Moreover, the appearing excess of calcium ions released from the dissolving calcium carbonate in Eq. (8) would keep mineral phosphate components within the solid phase by retarding reaction in Eq. (4), in accordance with the mass action law and/or binding the organic acid anions in a complex, such as that formed in Eq. (5), but without phosphate dissolution, as follows:



Corresponding experimental evidence was already reported at the beginning of the twentieth century. In the course of microbial release of acids, in soils rich in calcium carbonate, there would be only small quantities of phosphorus liberated (Kelley 1912; cited in Greaves 1922).

However, according to the experimental data of Szymkiewicz-Dabrowska et al. (2002), adding well-soluble ammonium or potassium bicarbonates (NH₄HCO₃, KHCO₃) or carbonates ((NH₄)₂CO₃, K₂CO₃) to soils mixed with AlPO₄·2H₂O, FePO₄·2H₂O, and Ca₃(PO₄)₂ resulted in increasing solubilities of the latter three minerals within a few days. This could be attributed to slowly ongoing hydrolysis

of the phosphates with the formation of basic salts, after the pH is raised in the presence of HCO_3^- and, especially, CO_3^{2-} anions owing to their hydrolysis. Specifically, surface hydrolysis reactions for Al (variscite) and Fe^{III} (strengite) phosphates occurring at higher pH values, releasing phosphate ions into solution and forming more basic insoluble metal phosphates, were described earlier (Bache 1963). Thus, as a result, a part of the phosphate anions in a mineral are substituted by OH^- ions within the solid phase and therefore become solubilized.

Overall discussion

Although the literature contains many reports on the use of TCP as a universal selection factor for PSB, empirical reports show that most of the strains isolated using this selector failed to deliver. Consequently, they were discarded and forgotten. Analysis of the literature and chemical considerations show that biological phosphate solubilization is a very complex phenomenon affected by numerous factors where each cannot be evaluated and tested separately. This happens because of the enormous possibilities presented by many soil types, insoluble phosphate species, and many potential PSB residing in these soils. Practically, it is not enough to know which organic acid is produced by the potential PSB and theoretically calculate how it functions to make insoluble P more soluble in the soils. Even though there are less soluble metal-P compounds than TCP, such as fluorapatite and hydroxyapatite, acid solubility knowledge of each compound alone, when tested in conjunction with the common knowledge of the main organic acids produced by plants or by the PSB, is not enough to predict with certainty what specific testing combination (organic acid–metal-P–plant–PSB) should be chosen. Too many, ever changing, chemical and later biological parameters are involved, and such theoretical prediction would not be reliable. Perhaps, this is the main reason why there are so many potential PSB isolated in vitro and such a low number of isolates that proved to be successful in inoculated plants. A practical strategy would be to test each PSB–plant interaction experimentally. Yet this would be feasible only when a very small number of isolates are selected in the first place. Isolation of PSB with TCP that produces numerous candidates is therefore not the best strategy and should be replaced.

Another common mistake prevailing in the literature is the measurement of growth promotion of plants inoculated by PSB in P-deficient soils as an indirect indicator for P solubilization. This assumption is based on the fact that many PGPB that are also PSB are known (Puente et al. 2004a, b, 2009a, b, and more). Yet the effect on plant growth, mostly on plant growth parameters, is not

necessarily related to phosphate solubilization, but rather to numerous other plant growth-promoting traits (Barret et al. 2011; Bashan and de-Bashan 2005, 2010; Lugtenberg and Kamilova 2009). A better indication that a potential PSB truly contributes to P content and metabolism of the plants is to evaluate P-related parameters of plant nutrition.

The most common laboratory test for P solubilization is the halo formation test known for half a century (Pikovskaya 1948). Here, the potential PSB grows on solid, rich medium in Petri dishes, where the sole P source is an insoluble P. Once a colony is growing, the solubilization process produces a halo, where the intensity of the solubilization is proportional to the size of the halo (Nautiyal 1999). Yet, many times, potential PSB are growing on these media without producing a visible halo, even after several transfers to the same medium (Puente et al. 2004a, b, 2009a, b; Lopez et al. 2011). This indicates that the importance of a halo, as a sole marker for P solubilization, is largely overestimated and is practically inadequate (see footnote a of Table 2).

Because current biological and chemical knowledge indicates that a universal selection factor for biological phosphate solubilization does not exist, the following conclusions and potential guidelines can be drawn:

- TCP, as a universal factor for isolating and evaluating PSB, is not a good selector according to much literature concerning failure with inoculated plants when using this compound for selection of PSB. Consequently, its use as sole selector should be abolished and the general technique should be complemented.
- There are several other common, insoluble metal-P compounds, some more insoluble than TCP, but none can replace it reliably for a universal selection factor because of chemical interactions.
- A combination of two to three metal-P compounds, when used together or in a tandem should replace the sole TCP as an initial selection factor. These combinations may or may not include TCP in the mix in alkaline soils.
- The selection of the metal-P candidates for potential PSB will depend on the type of soil (alkaline, acidic, or organic-rich) where the PSB will be used.
- Production of a halo on a solid agar medium should not be considered the sole test for P solubilization. When colonies grow without a halo after several replacements of the medium, an additional test in liquid media to assay P dissolution should be performed.
- The few bacterial isolates that are obtained after such rigorous selection should be further tested for abundant production of organic acids.
- Isolates complying with the above criteria should be tested on a model plant as *the ultimate test* for potential P solubilization.

- Parameters related to P nutrition in plants should be tested, not growth promotion in general.
- Consequently, we propose that new manuscripts that report initial isolation of “potential PSB” should not be considered for publication without exhaustive testing.

Acknowledgments We thank Prof. Hani Antoun from Laval University, Quebec, Canada for proposing the type of evaluations that were needed for this essay. Ira Fogel of CIBNOR provided editorial services. Preparation of this essay was supported by The Bashan Foundation, USA.

References

- Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol Res* 163:173–181
- Ahn PM (1993) Tropical soils and fertilizer use. Intermediate tropical agriculture series. Longman, Essex
- Alikhani HA, Saleh-Rastin N, Antoun H (2006) Phosphate solubilization activity of rhizobia native to Iranian soils. *Plant Soil* 287:35–41
- Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalonde R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). *Plant Soil* 204:57–67
- Arcand MM, Schneider KD (2006) Plant- and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: a review. *An Acad Bras Cienc* 78:791–807
- Babana AH, Antoun H (2005) Biological system for improving the availability of Tilemsi phosphate rock for wheat (*Triticum aestivum* L) cultivated in Mali. *Nutr Cycl Agroecosyst* 72:147–157
- Babana AH, Antoun H (2006) Effect of Tilemsi phosphate rock-solubilizing microorganisms on phosphorus uptake and yield of field-grown wheat (*Triticum aestivum* L) in Mali. *Plant Soil* 287:51–58
- Bache BW (1963) Aluminium and iron phosphate studies relating to soils. I. Solution and hydrolysis of variscite and strengite. *J Soil Sci* 14:113–123
- Baig KS, Arshad M, Shaharouna B, Khalid A, Ahmed I (2012) Comparative effectiveness of *Bacillus* spp. possessing either dual or single growth-promoting traits for improving phosphorus uptake, growth and yield of wheat (*Triticum aestivum* L.). *Ann Microbiol*. doi:10.1007/s13213-011-0352-0
- Bardiya MC, Gaur AC (1974) Isolation and screening of microorganisms dissolving low-grade rock phosphate. *Folia Microbiol* 19:386–389
- Barret M, Morrissey JP, O’Gara F (2011) Functional genomics analysis of plant-growth promoting rhizobacterial traits involved in rhizosphere competence. *Biol Fertil Soils* 47:729–743
- Bashan Y, de-Bashan LE (2005) Bacteria/Plant growth-promotion. In: Hillel D (ed) *Encyclopedia of soils in the environment*. Vol. 1. Elsevier, Oxford, pp 103–115
- Bashan Y, de-Bashan LE (2010) How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—a critical assessment. *Adv Agron* 108:77–136
- Bashan Y, Moreno M, Troyo E (2000) Growth promotion of the seawater-irrigated oil seed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* spp. *Biol Fertil Soils* 32:265–272
- Bationo A, Ayuk E, Ballo D, Kone M (1997) Agronomic and economic evaluation of Tilemsi phosphate rock in different agroecological zones of Mali. *Nutr Cycl Agroecosyst* 48:179–189
- Belimov AA, Safronova VI, Mimura T (2002) Response of spring rape (*Brassica napus* var. *oleifera* L.) to inoculation with plant growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant. *Can J Microbiol* 48:189–199
- Ben Farhat M, Farhat A, Bejar W, Kammoun R, Bouchaala K, Fourati A, Antoun H, Bejar S, Chouayekh H (2009) Characterization of the mineral phosphate solubilizing activity of *Serratia marcescens* CTM 50650 isolated from the phosphate mine of Gafsa. *Arch Microbiol* 191:815–824
- Brosheer JC, Lenfesty FA, Anderson JF Jr (1954) Solubility in the system aluminum phosphate–phosphoric acid–water. *J Am Chem Soc* 76:5951–5956
- Castagno LN, Estrella MJ, Sannazzaro AI, Grassano AE, Ruiz OA (2011) Phosphate-solubilization mechanism and in vitro plant growth promotion activity mediated by *Pantoea eucalypti* isolated from *Lotus tenuis* rhizosphere in the Salado River Basin (Argentina). *J Appl Microbiol* 110:151–165
- Cawthray GR (2003) An improved reversed-phase liquid chromatographic method for the analysis of low-molecular mass organic acids in plant root exudates. *J Chromatogr* 1011:233–240
- Chabot R, Antoun H, Cescas MP (1996) Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarium* biovar *phaseoli*. *Plant Soil* 184:311–321
- Chang C-H, Yang S-S (2009) Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. *Bioresour Technol* 100:1648–1658
- Chen YP, Rekha PD, Arunshen AB, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl Soil Ecol* 34:33–41
- Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biol Biochem* 37:1970–1974
- Collavino MM, Sansberro PA, Mroginski LA, Aguilar OM (2010) Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. *Biol Fertil Soils* 46:727–738
- de Freitas JR, Banerjee NR, Germida JJ (1997) Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). *Biol Fertil Soils* 24:358–364
- de-Bashan LE, Bashan Y (2004) Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). *Water Res* 38:4222–4246
- Dorozhkin SV (2009) Calcium orthophosphate-based biocomposites and hybrid biomaterials. *J Mater Sci* 44:2343–2387
- Dorozhkin SV (2011) Calcium orthophosphates. Occurrence, properties, biomineralization, pathological calcification and biomimetic applications. *Biomatter* 1:121–164
- Duffield JR, Edwards K, Evans DA, Morrish DM, Vobe RA, Williams DR (1991) Low molecular mass aluminum complex speciation in biofluids. *J Coord Chem* 23:277–290
- El-Tarabily KA, Youssef T (2010) Enhancement of morphological, anatomical and physiological characteristics of seedlings of the mangrove *Avicennia marina* inoculated with a native phosphate-solubilizing isolate of *Oceanobacillus picturatae* under greenhouse conditions. *Plant Soil* 332:147–162
- Evans WG, Pierce AG (1981) Calcium-phytate complex formation studies. *J Am Oil Chem Soc* 58:850–851

- Fankem H, Ngo Nkot L, Deubel A, Quinn J, Merbach W, Etoa F-X, Nwaga D (2008) Solubilization of inorganic phosphates and plant growth promotion by strains of *Pseudomonas fluorescens* isolated from acidic soils of Cameroon. *Afr J Microbiol Res* 2:171–178
- Fernández LA, Zalba P, Gómez MA, Sagardoy MA (2007) Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. *Biol Fertil Soils* 43:805–809
- Gerretsen FC (1948) The influence of microorganisms on the phosphate intake by the plant. *Plant Soil* 1:51–81
- Ghassemi M, Recht HL (1971) Phosphate precipitation with ferrous iron. Project No. 17010 EKI. US Environmental Protection Agency, Washington, DC, 69 pp
- Gillis MB, Edwards HM Jr, Young RJ (1962) Studies on the availability of calcium orthophosphates to chickens and turkeys. *J Nutr* 78:155–161
- Goldstein AH (1995) Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. *Biol Agric Hort* 12:185–193
- Goldstein AH (2007) Future trends in research on microbial phosphate solubilization: one hundred years of insolubility. In: Velázquez E, Rodríguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. *Developments in plant and soil sciences*, Vol. 102. Springer, Dordrecht, pp 91–96
- Goldstein AH, Krishnaraj PU (2007) Phosphate solubilizing microorganisms vs. phosphate mobilizing microorganisms: what separates a phenotype from a trait? In: Velázquez E, Rodríguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. *Developments in plant and soil sciences*, vol. 102. Springer, Dordrecht, pp 203–213
- Goldstein AH, Liu ST (1987) Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. *Biotechnology* 5:72–74
- Goldstein AH, Braverman K, Osorio N (1999) Evidence for mutualism between a plant growing in a phosphate-limited desert environment and a mineral phosphate solubilizing (MPS) rhizobacterium. *FEMS Microbiol Ecol* 30:295–300
- Greaves JE (1922) Influence of salts on bacterial activities of soil. *Bot Gaz* 73:161–180
- Grynspan F, Cheryan M (1983) Calcium phytate: effect of pH and molar ratio on in vitro solubility. *J Am Oil Chem Soc* 60:1761–1764
- Gulati A, Rahi P, Vyas P (2008) Characterization of phosphate-solubilizing fluorescent pseudomonads from the rhizosphere of seabuckthorn growing in the cold deserts of Himalayas. *Curr Microbiol* 56:73–79
- Gulati A, Sharma N, Vyas P, Sood S, Rahi P, Pathania V, Prasad R (2010) Organic acid production and plant growth promotion as a function of phosphate solubilization by *Acinetobacter rhizosphaerae* strain BIHB 723 isolated from the cold deserts of the trans-Himalayas. *Arch Microbiol* 192:975–983
- Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* 245:83–93
- Hameeda B, Harini G, Rupela OP, Wani SP, Reddy G (2008) Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. *Microbiol Res* 163:234–242
- Harris JN, New PB, Martin PM (2006) Laboratory tests can predict beneficial effects of phosphate-solubilising bacteria on plants. *Soil Biol Biochem* 38:1521–1526
- Haynes RJ (1982) Effects of liming on phosphate availability in acid soils. *Plant Soil* 68:289–308
- He Z, Ohno T, Cade-Menun BJ, Erich MS, Honeycutt CW (2006) Spectral and chemical characterization of phosphates associated with humic substances. *Soil Sci Soc Am J* 70:1741–1751
- Hill JE, Kysela D, Elimelech M (2007) Isolation and assessment of phytate-hydrolysing bacteria from the DelMarVa Peninsula. *Environ Microbiol* 9:3100–3107
- Hoffland E (1992) Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. *Plant Soil* 140:279–289
- Idriss EE, Makarewicz O, Farouk A, Rosner K, Greiner R, Bochow H, Richter T, Borriß R (2002) Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. *Microbiology* 148:2097–2109
- Illmer P, Schinner F (1995) Solubilization of inorganic calcium phosphates-solubilization mechanisms. *Soil Biol Biochem* 27:257–263
- Illmer P, Barbato A, Schinner F (1995) Solubilization of hardly-soluble AlPO₄ with P-solubilizing microorganisms. *Soil Biol Biochem* 27:265–270
- Iqbal U, Jamil N, Ali I, Hasnain S (2010) Effect of zinc-phosphate-solubilizing bacterial isolates on growth of *Vigna radiate*. *Ann Microbiol* 60:243–248
- Ishio S, Kuwahara M, Nakawaga H (1986) Conversion of AlPO₄-P to Fe-bound P in sea sediments. *B Jpn Soc Sci Fish* 52:901–911
- Jiang J-Q, Graham NJD (1998) Pre-polymerised inorganic coagulants and phosphorus removal by coagulation—a review. *Water SA* 24:237–244
- Johri JK, Surange S, Nautiyal CS (1999) Occurrence of salt, pH, and temperature-tolerant, phosphate-solubilizing bacteria in alkaline soils. *Curr Microbiol* 39:89–93
- Jones DL (1998) Organic acids in the rhizosphere—a critical review. *Plant Soil* 205:25–44
- Jones DL, Darrah PR (1994) Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant Soil* 166:247–257
- Jorquera MA, Hernandez MT, Rengel Z, Marschner P, Mora ML (2008) Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. *Biol Fertil Soils* 44:1025–1034
- Katiyar V, Goel R (2003) Solubilization of inorganic phosphate and plant growth promotion by cold tolerant mutants of *Pseudomonas fluorescens*. *Microbiol Res* 158:163–168
- Kelley WP (1912) The effects of calcium and magnesium carbonates on some biological transformations of nitrogen in soils. *Univ Calif Publ Agric Sci* 1:39–49
- Kim KY, McDonald GA, Jordan D (1997) Solubilization of hydroxyapatite by *Enterobacter agglomerans* and cloned *Escherichia coli* in culture medium. *Biol Fertil Soils* 24:347–352
- Kim KY, Jordan D, Krishnan HB (1998) Expression of genes from *Rahnella aquatilis* that are necessary for mineral phosphate solubilization in *Escherichia coli*. *FEMS Microb Lett* 159:121–127
- Kpoblekou AK, Tabatabai MA (1994) Effect of organic acids on release of phosphorus from phosphate rocks. *Soil Sci* 158:442–453
- Kucey RMN, Janzen HH, Leggett ME (1989) Microbially mediated increases in plant-available phosphorus. *Adv Agron* 42:199–228
- Kumar V, Narula N (1999) Solubilization of inorganic phosphates and growth emergence of wheat as affected by *Azotobacter chroococcum* mutants. *Biol Fertil Soils* 28:301–305
- Linu MS, Stephen J, Jisha MS (2009) Phosphate solubilizing *Gluconacetobacter* sp., *Burkholderia* sp. and their potential interaction with cowpea (*Vigna unguiculata* (L.) Walp.). *Int J Agri Res* 4:79–87
- Liu H, Wu XQ, Ren JH, Ye JR (2011) Isolation and identification of phosphobacteria in poplar rhizosphere from different regions of China. *Pedosphere* 21:90–97
- Lobartini JC, Tan KH, Pape C (1998) Dissolution of aluminum and iron phosphate by humic acids. *Commun Soil Sci Plant Anal* 29:535–544

- Lopez BR, Bashan Y, Bacilio M (2011) Endophytic bacteria of *Mammillaria fraileana*, an endemic rock-colonizing cactus of the Southern Sonoran Desert. *Arch Microbiol* 193:527–541
- Lopez BR, Tinoco-Ojanguren C, Bacilio M, Mendoza A, Bashan Y (2012) Endophytic bacteria of the rock-dwelling cactus *Mammillaria fraileana* affect plant growth and mobilization of elements from rocks. *Environ Exp Bot* 81:26–36
- Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* 63:541–556
- Mamta Rahi P, Pathania V, Gulati A, Singh B, Bhanwra RK, Tewari R (2010) Stimulatory effect of phosphate-solubilizing bacteria on plant growth, stevioside and rebaudioside-A contents of *Stevia rebaudiana* Bertoni. *Appl Soil Ecol* 46:222–229
- Martin RB (1997) The importance of aluminium chemistry for biological systems. In: Zatta PF, Alfrey AC (eds) Aluminium toxicity in infants' health and disease. World Scientific, Singapore, pp 3–15
- Mehta S, Nautiyal CS (2001) An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Curr Microbiol* 43:51–56
- Merbach W, Fankem H, Deubel A (2009) Influence of rhizosphere bacteria of African oil palm (*Elaeis guineensis*) on calcium, iron, and aluminum phosphate in vitro mobilization. In: International symposium "Root Research and Applications", 2–4 September 2009. BOKU, Vienna, Austria. URL: <http://asr.boku.ac.at/fileadmin/files/RRcd/session03/poster/042.pdf>
- Merbach W, Deubel A, Gransee A, Ruppel S, Klamroth A-K (2010) Phosphorus solubilization in the rhizosphere and its possible importance to determine phosphate plant availability in soil. A review with main emphasis on German results. *Arch Agron Soil Sci* 56(2):119–138
- Middleton VG (ed) (2003) Encyclopedia of sediments and sedimentary rocks. Encyclopedia of earth sciences series. Kluwer, Dordrecht
- Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Role of phosphatase enzymes in soil. In: Bunemann EK, Oberson A, Frossard E (eds) Phosphorus in action. Soil biology vol. 26. Springer, Berlin, pp 215–241
- Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol Lett* 170:265–270
- Naz I, Bano A (2010) Biochemical, molecular characterization and growth promoting effects of phosphate solubilizing *Pseudomonas* sp. isolated from weeds grown in salt range of Pakistan. *Plant Soil* 334:199–207
- Ogut M, Er F, Kandemir N (2010) Phosphate solubilization potentials of soil *Acinetobacter* strains. *Biol Fertil Soils* 46:707–715
- Oliveira CA, Alves VMC, Marriel IE, Gomes EA, Scotti MR, Carneiro NP, Guimarães CT, Schaffert RE, Sà NMH (2009) Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. *Soil Biol Biochem* 41:1782–1787
- Park K-H, Lee O-M, Jung H-I, Jeong J-H, Jeon Y-D, Hwang D-Y, Lee C-Y, Son H-J (2010) Rapid solubilization of insoluble phosphate by a novel environmental stress-tolerant *Burkholderia vietnamiensis* M6 isolated from ginseng rhizospheric soil. *Appl Microbiol Biotechnol* 86:947–955
- Peix A, Rivas R, Mateos PF, Martínez-Molina E, Rodríguez-Barrueco C, Velazquez E (2003) *Pseudomonas rhizosphaerae* sp. nov., a novel species that actively solubilizes phosphate in vitro. *Int J Syst Evol Microbiol* 53:2067–2072
- Peix A, Rivas R, Santa-Regina I, Mateos PF, Martínez-Molina E, Rodríguez-Barrueco C, Velazquez E (2004) *Pseudomonas lutea* sp. nov., a novel phosphate-solubilizing bacterium isolated from the rhizosphere of grasses. *Int J Syst Evol Microbiol* 54:847–850
- Pérez E, Sulbarán M, Ball MM, Yarzabal LA (2007) Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biol Biochem* 39:2905–2914
- Pikovskaya RI (1948) Mobilization of phosphates in soil in relation with vital activity of some microbial species. *Mikrobiologiya* 17:362–370 (in Russian)
- Puente ME, Bashan Y, Li CY, Lebsky VK (2004a) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. *Plant Biol* 6:629–642
- Puente ME, Li CY, Bashan Y (2004b) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. *Plant Biol* 6:643–650
- Puente ME, Li CY, Bashan Y (2009a) Rock-degrading endophytic bacteria in cacti. *Environ Exp Bot* 66:389–401
- Puente ME, Li CY, Bashan Y (2009b) Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. *Environ Exp Bot* 66:402–408
- Rajan SSS, Watkinson JH, Sinclair AG (1996) Phosphate rocks for direct application to soils. *Adv Agron* 57:77–159
- Rajapaksha RMCP, Herath D, Senanayake AP, Senevirathne MGTL (2011) Mobilization of rock phosphate phosphorus through bacterial inoculants to enhance growth and yield of wetland rice. *Commun Soil Sci Plant Anal* 42:301–314
- Rajkumar M, Nagendran R, Lee KJ, Lee WH, Kim SZ (2006) Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. *Chemosphere* 62:741–748
- Rengel Z, Marschner P (2005) Nutrient availability and management in the rhizosphere: exploiting genotypic differences. *New Phytol* 168:305–312
- Reyes I, Baziramakenga R, Bernier L, Antoun H (2001) Solubilization of phosphate rocks and minerals by a wild-type strain and two UV-induced mutants of *Penicillium rugulosum*. *Soil Biol Biochem* 33:1741–1746
- Reyes I, Valery A, Valduz Z (2006) Phosphate-solubilizing microorganisms isolated from rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. *Plant Soil* 287:69–75
- Richardson A (1994) Soil microorganisms and phosphorus availability. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) Soil biota: management in sustainable farming systems. CSIRO, Victoria, pp 50–62
- Richardson A (2001) Prospect for using soil microorganisms to improve the acquisition of phosphorus by plants. *Aust J Plant Physiol* 28:897–906
- Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17:319–339
- Rodríguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant Soil* 287:15–21
- Sackett WG, Patten AJ, Brown CV (1908) The solvent action of soil bacteria upon the insoluble phosphates of raw bonemeal and natural raw rock phosphate. *Centralbl Bakteriell* 202:688–703
- Schwab AP (1989) Manganese-phosphate solubility relationships in an acid soil. *Soil Sci Soc Am J* 53:1654–1660
- Selvakumar G, Joshi P, Nazim S, Mishra PK, Bisht JK, Gupta HS (2009) Phosphate solubilization and growth promotion by *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium isolated from a high altitude Himalayan rhizosphere. *Biologia* 64:239–245
- Son H-J, Park G-T, Cha M-S, Heo M-S (2006) Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizosphere. *Bioresour Technol* 97:204–210
- Song O-R, Lee S-J, Lee Y-S, Lee S-C, Kim K-K, Choi Y-L (2008) Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA23 isolated from cultivated soil. *Braz J Microbiol* 39:151–156

- Srinivasan R, Alagawadi AR, Yandigeri MS, Meena KK, Saxena AK (2012) Characterization of phosphate-solubilizing microorganisms from salt-affected soils of India and their effect on growth of sorghum plants [*Sorghum bicolor* (L.) Moench]. *Ann Microbiol* 62:93–105
- Stumm W, Morgan JJ (1996) *Aquatic chemistry*, 3rd edn. Wiley, New York
- Szymkiewicz-Dabrowska D, Lachacz A, Huszcza-Ciolkowska G (2002) The contribution of soil solution CO_2 (HCO_3^-) to incorporation of sparingly soluble phosphates to the pool of phosphates available for plants. *Zeszyty Problemowe Postepow Nauk Rolniczych*, no. 484, pp. 701–709 (URL: <http://psjc.icm.edu.pl/psjc/cgi-bin/getdoc.cgi?AAAA00721>)
- Taurian T, Anzuay MS, Angelini JG, Tonelli ML, Ludueña L, Pena D, Ibáñez F, Fabra A (2010) Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. *Plant Soil* 329:421–431
- Trivedi P, Sa T (2008) *Pseudomonas corrugata* (NRRL B-30409) mutants increased phosphate solubilization, organic acid production, and plant growth at lower temperatures. *Curr Microbiol* 56:140–144
- Unno Y, Okubo K, Wasaki J, Shinano T, Osaki M (2005) Plant growth promotion abilities and microscale bacterial dynamics in the rhizosphere of lupin analysed by phytate utilization ability. *Environ Microbiol* 7:396–404
- Vassilev N, Toro M, Vassileva M, Azcon R, Barea JM (1997) Rock phosphate solubilization by immobilized cells of *Enterobacter* sp. in fermentation and soil conditions. *Bioresour Technol* 61:29–32
- Vazquez P, Holguin G, Puente ME, Lopez-Cortes A, Bashan Y (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol Fertil Soils* 30:460–468
- Viruel E, Lucca ME, Siñeriz F (2011) Plant growth promotion traits of phosphobacteria isolated from Puna, Argentina. *Arch Microbiol* 193:489–496
- Wang L, Nancollas GH (2008) Calcium orthophosphates: crystallization and dissolution. *Chem Rev* 108:4628–4669
- Welch SA, Taunton AE, Banfield JF (2002) Effect of microorganisms and microbial metabolites on apatite dissolution. *Geomicrobiol J* 19:343–367
- Whitelaw MA (1999) Growth promotion of plants inoculated with phosphate-solubilizing fungi. *Adv Agron* 69:99–151
- Xiang W-L, Liang H-Z, Liu S, Luo F, Tang J, Li M-Y, Che Z-M (2011) Isolation and performance evaluation of halotolerant phosphate solubilizing bacteria from the rhizospheric soils of historic Dagong brine well in China. *World J Microbiol Biotechnol* 27:2629–2637
- Xiao CQ, Chi RA, Li WS, Zheng Y (2011) Biosolubilization of phosphorus from rock phosphate by moderately thermophilic and mesophilic bacteria. *Miner Eng* 24:956–958
- Yu X, Liu X, Zhu TH, Liu GH, Mao C (2011) Isolation and characterization of phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. *Biol Fertil Soils* 47:437–446
- Zou K, Binkley D, Doxtader KG (1992) A new method for estimating gross phosphorus mineralization and immobilization rates in soils. *Plant Soil* 147:243–250