ELSEVIER

Biotechnology Advances 17 (1999) 319–339

BIOTECHNOLOGY ADVANCES

Research review paper

Phosphate solubilizing bacteria and their role in plant growth promotion

Hilda Rodríguez *, Reynaldo Fraga

Department of Microbiology, Cuban Research Institute on Sugarcane By-Products (ICIDCA), P.O. Box 4026, CP 11 000, Havana, Cuba

Abstract

The use of phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers. The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorous in soil. Several phosphatase-encoding genes have been cloned and characterized and a few genes involved in mineral phosphate solubilization have been isolated. Therefore, genetic manipulation of phosphate-solubilizing bacteria to improve their ability to improve plant growth may include cloning genes involved in both mineral and organic phosphate solubilization, followed by their expression in selected rhizobacterial strains. Chromosomal insertion of these genes under appropriate promoters is an interesting approach.

Keywords: Phosphate solubilization; Soil bacteria; Plant-growth-promoting bacteria; Rhizobacteria; Phosphatases; Biofertilization

1. Introduction

It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. Therefore, their use as biofertilizers or control agents for agriculture improvement has been a focus of numerous researchers for a number of years [1–5]. This group of bacteria has been termed ‘plant growth promoting rhizobacteria’ (PGPR) [6], and among them are strains from genera such as *Pseudomonas, Azospirillum, Burkholderia, Bacillus, Enterobacter, Rhizobium, Erwinia, Serratia, Alcaligenes, Arthrobacter, Acinetobacter* and *Flavobacterium.*

Stimulation of different crops by PGPR has been demonstrated in both laboratory and field trials. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* have increased

* Corresponding author. Fax: +53-7-338236; e-mail: icidca@ceniai.inf.cu

PII: S0734-9750(99)00014-2

root and shoot elongation in canola, lettuce, and tomato [7,8] as well as crop yields in potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants, and wheat [1,3,4,9]. Wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculants, [10] and a 10–20% yield increase in the same crop was reported in field trials using a combination of *Bacillus megaterium* and *Azotobacter chroococcum* [11]. *Azospirillum* spp. have increased yield in maize, sorghum, and wheat [12–14], and *Bacillus* spp. has increased yield in peanut, potato, sorghum, and wheat [15–17].

Bacterial inoculants have been used to increase plant yields in several countries, and commercial products are currently available. For example, in Cuba, several biofertilizers are commercially produced and employed with different crops, mostly using strains of *Azotobacter, Rhizobium, Azospirillum* and *Burkholderia.*

The mechanisms by which PGPR can exert a positive effect on plant growth can be of two types: direct and indirect [5]. Indirect growth promotion is the decrease or prevention of deleterious effect of pathogenic microorganisms, mostly due to the synthesis of antibiotics [18] or siderophores [19] by the bacteria. Direct growth promotion can be through the synthesis of phytohormones [20], N_2 fixation [21], reduction of membrane potential of the roots [22], synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones [23], as well as the solubilization of inorganic phosphate and mineralization of organic phosphate, which makes phosphorous available to the plants [24–26]. The occurrence of this last mechanism in several PGPRs and its possible role in the overall effects on plant growth promotion will be discussed in this review.

2. Phosphate availability in soil

Phosphorus (P) is one of the major essential macronutrients for biological growth and development [27]. It is present at levels of 400–1200 $mg \cdot kg^{-1}$ of soil [28]. Its cycle in the biosphere can be described as ‘open’ or ‘sedimentary’, because there is no interchange with the atmosphere [29]. Microorganisms play a central role in the natural phosphorus cycle. This cycle occurs by means of the cyclic oxidation and reduction of phosphorus compounds, where electron transfer reactions between oxidation stages range from phosphine (-3) to phosphate ($+5$). The genetic and biochemical mechanisms of these transformations are not yet completely understood [30].

The concentration of soluble P in soil is usually very low, normally at levels of 1 ppm or less (10 M $H_2PO_4^-$) [31]. The cell might take up several P forms but the greatest part is absorbed in the forms of HPO_4^{2-} or $H_2PO_4^-$ [32].

The biggest reserves of phosphorus are rocks and other deposits, such as primary apatites and other primary minerals formed during the geological age [28,33]. For example, it is estimated that there are almost 40 million tons of phosphatic rock deposits in India [34], and this material should provide a cheap source of phosphate fertilizer for crop production [35]. Mineral forms of phosphorus are represented in soil by primary minerals, such as apatite, hydroxyapatite, and oxyapatite. They are found as part of the stratum rock and their principal characteristic is their insolubility. In spite of that, they constitute the biggest reservoirs of this element in soil because, under appropriate conditions, they can be solubilized and become available for plants and microorganisms. Mineral phosphate can be also found associated with the surface of hydrated oxides of Fe, Al, and Mn, which are poorly soluble and assimilable. This is characteristic of ferralitic soils, in which hydration and accumulation of hydrated oxides and hydroxides of Fe takes place, producing an increase of phosphorus fixation capacity [28].

Most agricultural soils contain large reserves of phosphorus, a considerable part of which has accumulated as a consequence of regular applications of P fertilizers [36]. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants [37]. The phenomena of fixation and precipitation of P in soil is generally highly dependent on pH and soil type. Thus, in acid soils, phosphorus is fixed by free oxides and hydroxides of aluminum and iron, while in alkaline soils it is fixed by calcium, causing a low efficiency of soluble P fertilizers, such as super calcium [31,38,39]. According to Lindsay [40], superphosphate contains a sufficient amount of calcium to precipitate half of its own P, in the form of dicalcium phosphate or dicalcium phosphate dihydrated.

A second major component of soil P is organic matter. Organic forms of P may constitute 30–50% of the total phosphorus in most soils, although it may range from as low as 5% to as high as 95% [41]. Organic P in soil is largely in the form of inositol phosphate (soil phytate). It is synthesized by microorganisms and plants and is the most stable of the organic forms of phosphorus in soil, accounting for up to 50% of the total organic P [42–44]. Other organic P compounds in soil are in the form of phosphomonoesters, phosphodiesters including phospholipids and nucleic acids, and phosphotriesters.

Among identifiable components in hydrolysates of soil extracts are cytosine, adenine, guanine, uracil, hypoxanthine, and xanthine (decomposition products of guanine and adenine). Of the total organic phosphorus in soil, only approximately 1% can be identified as nucleic acids or their derivatives [41]. Among the phospholipids, choline has been identified as one of the products of the hydrolysis of lecithin. Various studies have shown that only approximately 1–5 ppm of phospholipids phosphorus occur in soil, although values as high as 34 ppm have been detected [41].

Many of these P compounds are high molecular-weight material which must first be bioconverted to either soluble ionic phosphate (Pi, HPO_4^{2-}, $H_2PO_4^{-}$), or low molecular-weight organic phosphate, to be assimilated by the cell [31]. Besides this, large quantities of xenobiotic phosphonates, which are used as pesticides, detergent additives, antibiotics, and flame retardants, are released into the environment. These C-P compounds are generally resistant to chemical hydrolysis and biodegradation, but recently several reports have documented microbial P release from these sources [30,45,46].

3. Phosphate solubilizing bacteria

3.1. Mineral phosphate solubilization

Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate [38]. Among the bacterial genera with this capacity are

Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium and *Erwinia.*

There are considerable populations of phosphate-solubilizing bacteria in soil and in plant rhizospheres [47–50]. These include both aerobic and anaerobic strains, with a prevalence of aerobic strains in submerged soils [49]. A considerably higher concentration of phosphate-solubilizing bacteria is commonly found in the rhizosphere in comparison with nonrhizosphere soil [48,49].

Visual detection and even semiquantitative estimation of the phosphate solubilization ability of microorganisms have been possible using plate screening methods, which show clearing zones around the microbial colonies in media containing insoluble mineral phosphates (mostly tricalcium phosphate or hydroxyapatite) as the single P source. In some cases, there have been contradictory results between plate halo detection and P solubilization in liquid cultures [51–53]. However, the method can be regarded as generally reliable for isolation and preliminary characterization of phosphate-solubilizing microorganisms [48,54–57]. Gupta et al. [58] developed an improved procedure using a medium containing bromophenol blue. In this medium, yellow colored halos are formed around the colonies in response to the pH drop produced by the release of organic acids, which are responsible for phosphate solubilization. With this method, the authors reported more reproducible and correlated results than with the simple halo method.

In vitro studies of the dynamics of phosphate solubilization by bacterial strains have been carried out based on the measurement of P release into culture broth, from cultures developed using an insoluble compound as the only P source. The rate of P solubilization is often estimated by subtracting the final P concentration (minus that of an inoculated control) from the initial theoretical P supplied by the P substrate. This estimation has the disadvantage of not taking into account the P utilized by the cells during growth.

Babenko et al. [59] have isolated and grouped phosphate-solubilizing bacteria into four different types, according to kinetics and rate of P accumulation. These groups range from a linear increase of P concentration along with the growth of the culture, to oscillating behavior with variations in the soluble P levels giving rise to several peaks and troughs of P concentration. This last type of kinetic behavior has also been observed [56,60,61]. These changes in P concentration could be a consequence of P precipitation of organic metabolites [59,60] and/or the formation of organo-P compounds with secreted organic acids, which are subsequently used as an energy or nutrient source, this event being repeated several times in the culture [56]. An alternative explanation could be the difference in the rate of P release and uptake. When the rate of uptake is higher than that of solubilization, a decrease of P concentration in the medium could be observed. When the uptake rate decreases (for instance as a consequence of decreasing growth or entry into stationary phase), the P level in the medium increases again. More probably, a combination of two or more phenomena could be involved in this behavior. Thus, the P concentration in the culture broth as an indication of phosphate solubilization capacity should be viewed with caution, and a kinetic study of this parameter would offer a more reliable picture of cellular behavior toward P.

The physiology of phosphate solubilization has not been studied thoroughly. Some studies indicate that certain mineral elements play a role in this process. A critical K concentration is necessary for optimum solubilization rates [32,56], while Mg and Na seem to be important in some fungi [32] but not in *Pseudomonas* strains [56]. The role of N and P uptake remains controversial [56,62].

Instability of the phosphate-solubilizing character of some strains after several cycles of inoculation has been reported [35,56,63]. However, the trait seems to remain stable in most isolates [64].

Table 1 summarizes the solubilization ability of different insoluble P substrates by several bacterial species. Although no accurate quantitative comparison can be made from experiments from different sources, the data suggest that *Rhizobium*, *Pseudomonas* and *Bacillus* species are among the most powerful solubilizers, while tricalcium phosphate and hydroxyapatite seem to be more degradable substrates than rock phosphate.

3.2. Organic phosphate solubilization

As discussed previously, soil contains a wide range of organic substrates, which can be a source of P for plant growth. To make this form of P available for plant nutrition, it must be hydrolyzed to inorganic P. Mineralization of most organic phosphorous compounds is carried out by means of phosphatase enzymes. The presence of a significant amount of phosphatase activity in soil has been reported [65–70]. Important levels of microbial phosphatase activity have been detected in different types of soils [71,72]. In fact, the major source of phosphatase activity in soil is considered to be of microbial origin [73,74]. In particular, phosphatase activity is substantially increased in the rhizosphere [75].

The presence of organic phosphate-mineralizing bacteria in soil has been surveyed by Greaves and Webley [76] for the rhizosphere of pasture grasses, by Raghu and MacRae [49] for rice plants, as well as by Bishop et al. [67] and Abd-Alla [77], and others.

The pH of most soils ranges from acidic to neutral values. Thus, acid phosphatases should play the major role in this process. Significant acid phosphatase activity was observed in the

Table 1
Total P accumulation in cultures of different bacterial species grown on insoluble mineral phosphate substrates (mg l^{-1})

Bacterial strain	Substrate			Reference
	$Ca_3(PO_4)_2$	Hydroxyapatite	Rock phosphate	
Pseudomonas sp.	52	nd	nd	[56]
Pseudomonas striata	156	143	22	[64]
Burkholderia cepacia	35	nd	nd	[61]
Rhizobium sp.	nd	300	nd	[115]
Rhizobium meliloti	nd	165	nd	[115]
Rhizobium leguminosarum	nd	356	nd	[115]
Rhizobium loti	nd	27	nd	[115]
Bacillus amyloliquefaciens	395	nd	nd	[121]
Bacillus polymyxa	116	87	17	[64]
Bacillus megaterium	82	31	16	[64]
Bacillus pulvifaciens	54	65	13	[64]
Bacillus circulans	11	17	6	[64]
Citrobacter freundi	16	7	5	[64]

nd indicates not determined.

rhizosphere of slash pine in two forested Spodosoils [78]. Burns [79] studied the activity of various phosphatases in the rhizosphere of maize, barley, and wheat, showing that phosphatase activity was considerable in the inner rhizosphere at acidic and neutral soil pH. Soil bacteria expressing a significant level of acid phosphatases include strains from the genus *Rhizobium* [77], *Enterobacter, Serratia, Citrobacter, Proteus* and *Klebsiella* [80], as well as *Pseudomonas* [81] and *Bacillus* [82].

According to Greaves and Webley [76], approximately 30–48% of culturable soil and rhizosphere microorganisms utilize phytate. On the other hand, Richardson and Hadobas [83] reported that 63% of culturable soil bacteria were able to grow on this substrate as carbon and P source on agar medium. However, of these, only 39–44% could utilize phytate as a P source in liquid medium, while a very low proportion could use it as a C source in this condition.

All of these studies provide evidence that support the role of bacteria in rendering organic P available to plants [84]. Some examples of soil bacteria capable of P release from different organic sources are shown in Table 2.

3.3. Phosphate-solubilizing bacteria as plant growth promoters

Although several phosphate solubilizing bacteria occur in soil, usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Thus, the amount of P liberated by them is generally not sufficient for a substantial increase in in situ plant growth. Therefore, inoculation of plants by a target microorganism at a much higher concentration than that normally found in soil is necessary to take advantage of the property of phosphate solubilization for plant yield enhancement.

There have been a number of reports on plant growth promotion by bacteria that have the ability to solubilize inorganic and/or organic P from soil after their inoculation in soil or plant seeds [9,25,26,85–88]. The production by these strains of other metabolites beneficial to the plant, such as phytohormones, antibiotics, or siderophores, among others, has created confusion about the specific role of phosphate solubilization in plant growth and yield stimulation

Table 2
Phosphate mineralization from P-substrates by some soil bacterial species

Bacterial strain	Substrate	Enzyme type	Reference
Pseudomonas fluorescens	Non-specific	Acid phosphatase	[81]
Pseudomonas sp.	Non-specific	Acid phosphatase	[81]
Burkholderia cepacia	Non-specific	Acid phosphatase	[61]
Enterobacter aerogenes	Non-specific	Acid phosphatase	[80]
Enterobacter cloacae	Non-specific	Acid phosphatase	[80]
Citrobacter freundi	Non-specific	Acid phosphatase	[80]
Proteus mirabalis	Non-specific	Acid phosphatase	[80]
Serratia marcenscens	Non-specific	Acid phosphatase	[80]
Bacillus subtilis	Inositol phosphate	Phytase	[83]
Pseudomonas putida	Inositol phosphate	Phytase	[83]
Pseudomonas mendocina	Inositol phosphate	Phytase	[83]
Pseudomonas fluorescens	Phosphonoacetate	Phosphonoacetate hydrolase	[45]
Bacillus licheniformis	D-α-glycerophosphate	D-α-glycerophosphatase	[82]
Klebsiella aerogenes	Phosphonates	C-P Lyase	[30]

[1,10]. However, at present, there is evidence supporting the role of this mechanism in plant growth enhancement. For example, several soil microorganisms, including bacteria, improve the supply of P to plants as a consequence of their capability for inorganic or organic P solubilization [24,36,89]. Considering that P availability is a limiting step in plant nutrition [38], this evidence suggests a fundamental contribution of phosphate-solubilizing bacteria to plant nutrition and, therefore, to the improvement of plant growth performance.

Chabot et al. [90] demonstrated growth stimulation of maize and lettuce by several microorganisms capable of mineral phosphate solubilization. A strain of *Burkholderia cepacia* showing no indoleacetic acid production, but displaying significant mineral phosphate solubilization and moderate phosphatase activity [61] has improved the yield of tomato, onion, potato, banana, citrics, and coffee, among other cultivars, in field tests, and is currently being used as a commercial biofertilizer in Cuba (Martínez A. et al., personal communication).

Furthermore, several examples of simultaneous growth promotion and increase in P uptake by plants as the result of phosphate-solubilizing bacteria inoculations have been reported. Inoculation with two strains of *Rhizobium leguminosarum* selected for their P-solubilization ability has been shown to improve root colonization and growth promotion and to increase significantly the P concentration in lettuce and maize [91,92]. Chabot et al. concluded that the phosphate-solubilization effect of *Rhizobia* and other mineral phosphate-solubilizing microorganisms seems to be the most important mechanism of plant growth promotion in moderately fertile and very fertile soils. On the other hand, a strain of *Pseudomonas putida* also stimulated the growth of roots and shoots and increased ^{32}P-labeled phosphate uptake in canola [89]. Inoculation of rice seeds with *Azospirillum lipoferum* strain 34H increased the phosphate ion content and resulted in significant improvement of root length and fresh and dry shoot weights [93]. Simultaneous increases in P uptake and crop yields have also been observed after inoculation with *Bacillus firmus* [87], *Bacillus polymyxa* [25] and *Bacillus cereus* [94], and others.

An alternative approach for the use of phosphate-solubilizing bacteria as microbial inoculants is the use of mixed cultures or co-inoculation with other microorganisms. Several studies demonstrate the beneficial influence of combined inoculation of phosphate-solubilizing bacteria and *Azotobacter* on yield, as well as on nitrogen (N) and P accumulation in different crops [95,96]. Co-inoculation of *Pseudomonas striata* and *Bacillus polymyxa* strains showing phosphate-solubilizing ability, with a strain of *Azospirillum brasilense*, resulted in a significant improvement of grain and dry matter yields, with a concomitant increase in N and P uptake, compared with separate inoculations with each strain [97]. Also, phosphate-solubilizing *Agrobacterium radiobacter* combined with nitrogen fixer *Azospirillum lipoferum* produced improved grain yield of barley compared with single inoculations in pot and field experiments [98]. These authors concluded that mixed inoculants provided more balanced nutrition for the plants, and that the improvement in N and P uptake was the major mechanism involved. This evidence points to the advantage of the mixed inoculations of PGPR strains comprising phosphate-solubilizing bacteria.

On the other hand, it has been postulated that some phosphate-solubilizing bacteria behave as mycorrhizal helper bacteria [99,100]. In this regard, several studies have shown that phosphate-solubilizing bacteria interact with vesicular arbuscular mycorrhizae (VAM) by releasing phosphate ions in the soil, which causes a synergistic interaction that allows for better exploitation of poorly soluble P sources [101–103]. It is likely that the phosphate solubilized by the bacteria could be more efficiently taken up by the plant through a mycorrhizae-mediated bridge between roots and surrounding soil that allows nutrient translocation from soil to plants [104]. In fact, Toro et al. [105], using radioactive ^{32}P labeling, demonstrated that phosphate-solubilizing bacteria associated with VAM improved mineral (N and P) accumulation in plant tissues. These authors suggested that the inoculated rhizobacteria could have released phosphate ions from insoluble rock phosphate and/or other P sources, which were then taken up by the external VAM mycelium.

Commercial biofertilizers claiming to undergo phosphate solubilization using mixed bacterial cultures have been developed. Examples of these are: 'Phylazonit-M' (permission at No. 9961, 1992, by the Ministry of Agriculture of Hungary), a product containing *Bacillus megaterium*; *Azotobacter chroococcum*, which allows an increase in N and P supply to the plants; and the product known as 'KYUSEI EM' (EM Technologies, Inc.), a mixed inoculum including lactic acid bacteria, the lactic acid being the agent for mineral phosphate solubilization.

Considerable evidence supports the specific role of phosphate solubilization in the enhancement of plant growth by phosphate-solubilizing bacteria. However, not all laboratory or field trials have offered positive results. For example, an inoculant using *Bacillus megaterium* var. *phosphoricum*, was applied successfully in the former Soviet Union and India, but it did not show the same efficiency in soils in the United States [106]. Undoubtedly, the efficiency of the inoculation varies with the soil type, specific cultivar, and other parameters. The P content of the soil is probably one of the crucial factors in determining the effectiveness of the product.

4. Mechanisms of phosphate solubilization

4.1. Solubilization of mineral phosphates

It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms [35,107–112]. Production of organic acids results in acidification of the microbial cell and its surroundings. Consequently, Pi may be released from a mineral phosphate by proton substitution for Ca^{2+} [31]. The production of organic acids by phosphate solubilizing bacteria has been well documented. Among them, gluconic acid seems to be the most frequent agent of mineral phosphate solubilization. It is reported as the principal organic acid produced by phosphate solubilizing bacteria such as *Pseudomonas* sp. [56], *Erwinia herbicola* [113], *Pseudomonas cepacia* [114] and *Burkholderia cepacia* (Rodriguez et al., unpublished results). Another organic acid identified in strains with phosphate-solubilizing ability is 2-ketogluconic acid, which is present in *Rhizobium leguminosarum* [35], *Rhizobium meliloti* [115], *Bacillus firmus* [109], and other unidentified soil bacteria [107]. Strains of *Bacillus liqueniformis* and *Bacillus amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids. Other organic acids, such as glycolic, oxalic, malonic, and succinic acid, have also been identified among phosphate solubilizers [56,109].

There is also experimental evidence that supports the role of organic acids in mineral phosphate solubilization. Halder et al. [35] showed that the organic acids isolated from a culture of *Rhizobium leguminosarum* solubilized an amount of P nearly equivalent to the amount that was solubilized by the whole culture. Besides this, treatment of the culture filtrates from several *Rhizobium* strains with pepsin or removal of proteins by acetone precipitation did not affect phosphate release capacity, showing that this was not an enzymatic process. However, neutralization with NaOH destroyed the solubilization activity [115]. Based on these findings, following the cloning of mineral phosphate solubilization genes, Goldstein [31,116] has proposed that the direct periplasmic oxidation of glucose to gluconic acid, and often 2-ketogluconic acid, forms the metabolic basis of the mineral phosphate solubilization phenotype in some Gram negative bacteria.

Alternative possibilities other than organic acids for mineral phosphate solubilization have been proposed based on the lack of a linear correlation between pH and the amount of solubilized P [27,117,118]. In addition, no significant amounts of organic acid production could be detected from a phosphate solubilizer fungus, *Penicillium* sp. [56]. Studies have shown that the release of H^+ to the outer surface in exchange for cation uptake or with the help of H^+ translocation ATPase could constitute alternative ways for solubilization of mineral phosphates.

Other mechanisms have been considered, such as the production of chelating substances by microorganisms [47,107] as well as the production of inorganic acids, such as sulphidric [47,119], nitric, and carbonic acid [120]. However, the effectiveness of these processes has been questioned and their contribution to P release in soil appears to be negligible [119,121].

4.2. Mineralization of organic phosphorus

Organic phosphate solubilization is also called mineralization of organic phosphorus, and it occurs in soil at the expense of plant and animal remains, which contain a large amount of organic phosphorus compounds. The decomposition of organic matter in soil is carried out by the action of numerous saprophytes, which produce the release of radical orthophosphate from the carbon structure of the molecule. The organophosphonates can equally suffer a process of mineralization when they are victims of biodegradation [45]. The microbial mineralization of organic phosphorus is strongly influenced by environmental parameters; in fact, moderate alkalinity favors the mineralization of organic phosphorus [41].

The degradability of organic phosphorous compounds depends mainly on the physicochemical and biochemical properties of their molecules, e.g. nucleic acids, phospholipids, and sugar phosphates are easily broken down, but phytic acid, polyphosphates, and phosphonates are decomposed more slowly [30,45,46].

The mineralization of these compounds is carried out by means of the action of several phosphatases (also called phosphohydrolases). These dephosphorylating reactions involve the hydrolysis of phosphoester or phosphoanhydride bonds. The phosphohydrolases are clustered in acid or alkaline. The acid phosphohydrolases, unlike alkaline phosphatases, show optimal catalytic activity at acidic to neutral pH values. Moreover, they can be further classified as specific or nonspecific acid phosphatases, in relation to their substrate specificity. Rossolini et al. [122] recently published a comprehensive review of bacterial nonspecific acid phosphohydrolases. The specific phosphohydrolases with different activities include: 3′-nucleotidases and 5′-nucleotidases [123]; hexose phosphatases [124]; and phytases [125]. A specific group of P releasing enzymes are those able to cleave C-P bonds from organophosphonates [30,45,46,126].

Some phosphohydrolases are secreted outside the plasma membrane, where they are either released in a soluble form or retained as membrane-bound proteins. This localization allows them to act as scavenging enzymes on organic phosphoesters that are components of high molecular weight material (i.e. RNA and DNA) and cannot cross the cytoplasmic membrane. This material can be first converted to low molecular weight components, and this process may occur sequentially i.e. the transformation of RNA and DNA to nucleoside monophosphate via RNase and DNase respectively, followed by the release of P and organic by-products via phosphohydrolases, providing the cell with essential nutrients [31].

5. Genetics of phosphate solubilizing bacteria

5.1. Genetics of mineral phosphate solubilization

The genetic basis of mineral phosphate solubilization (i.e. the Mps^+ phenotype) [57] is not well understood. Because the production of organic acids is considered to be the principal mechanism for mineral phosphate solubilization, it could be assumed that any gene involved in organic acid synthesis might have an effect on this character.

Goldstein and Liu [57] cloned a gene from *Erwinia herbicola* that is involved in mineral phosphate solubilization by screening the antibiotic-resistant recombinants from a genomic library in a medium containing hydroxyapatite as the source of P. The expression of this gene allowed production of gluconic acid and mineral phosphate solubilization activity in *E. coli* HB101. Sequence analysis of this gene [113] suggested its probable involvement in the synthesis of the enzyme pyrroloquinoline quinone (PQQ) synthase, which directs the synthesis of PQQ, a co-factor necessary for the formation of the holoenzyme glucose dehydrogenase (GDH)-PQQ. This enzyme catalyzes the formation of gluconic acid from glucose by the direct oxidation pathway.

Following a similar strategy, a mineral phosphate solubilization gene from *Pseudomonas cepacia* was isolated [127]. This gene (*gabY*), whose expression also allowed the induction of the mineral phosphate solubilization phenotype via gluconic acid production in *Escherichia coli* JM109, showed no apparent homology with the previous cloned PQQ synthetase gene [113,128], but it did with a permease system membrane protein. The *gabY* gene could play an alternative role in the expression and/or regulation of the direct oxidation pathway in *Pseudomonas cepacia*, thus acting as a functional mineral phosphate solubilization gene in vivo.

Very little is known regarding the genetic regulation governing the mineral phosphate solubilization trait. In fact, the information about the genetic or biochemical mechanisms involved in the synthesis of the GDH-PQQ holoenzyme is scant, and variations between constitutive and inducible phenotypes are observed among several bacterial species [31]. Glucose, gluconate, manitol, and glycerol are among the possible inducers of the holoenzyme activity [129].

Concerning the possible effect of soluble P on the expression of the phosphate-solubilizing activity, Goldstein and Liu [57] found that the mineral phosphate solubilization trait in *E. herbicola* is induced by P starvation and repressed by elevated exogenous P levels (complete repression achieved at P concentrations $>$20 mM). Coincidentally, a *Burkholderia cepacia* strain showed reduced expression of tricalcium phosphate solubilization at increasing phosphate concentration >2 mM, and finally failed to express any solubilization ability at P levels between 30 and 40 mM (Rodríguez, unpublished results). However, Halder et al. [35] found no effect of soluble P up to 6 mM on rock phosphate solubilization in cultures of *Rhizobium leguminosarum.* Mikanova et al. [130] isolated a number of phosphate solubilizing bacteria, some of them exhibiting repression of this trait under the presence of soluble P and others showing no repression effect at concentrations up to 50 mM. These data thus suggest that P availability could regulate mineral phosphate solubilization in some species and have no effect in others. This aspect needs to be investigated in more detail, in particular for soil bacterial isolates.

6. Genetics of organic phosphate mineralization

Different patterns of phosphatase activity are widespread in bacteria, particularly in those belonging to the family *Enterobacteriaceae.* The production of these enzymes is often controlled by complex regulatory mechanisms, so that the enzyme activity is detectable only under specific environmental conditions. In fact, a comprehensive understanding of the properties, regulation, and role of these enzymes is still lacking; even in *Escherichia coli* and *Salmonella typhimurium,* which are the most thoroughly investigated in this regard [131], only some genes have been cloned, sequenced, and studied for their effects on regulation.

The principal mechanism for the regulation of phosphatases production is the regulation by inorganic phosphate (Pi) concentration (i.e. phosphate-repressible phosphatases). This mechanism has been best studied in the alkaline phosphatase (gene *phoA*) of *E. coli*, which is suddenly and fully induced when the Pi concentration decreases from 100 mM to 0.16 mM [132]. The mechanism involves a Pi transport operon as a regulatory element, in addition to the sensor-activator operon. The genes controlled by Pi and activated by PhoB constitute the PHO regulon [133].

Another Pi repressible bacterial phosphatase is the alkaline phosphatase of *Morganella morganii*, produced under conditions of low-Pi availability, which, according to its regulation and the molecular mass of its polypeptide components, is probably similar to that of *E. coli* [134]. *Pseudomonas fluorescens* MF3, *Providencia stuartii*, and *Providencia rettgeri* also produce alkaline phosphatase activity, which is repressed by phosphate [80,81]. Some authors suggest that the regulation of the expression of phosphatase genes in other genus belonging to the family *Enterobacteriaceae* may be similar to the *pho* genes from *E. coli*, based on the high degree of conservation of the promoter structure between these genes. For example, the sequence in the –35 region of *phoC* (encoding for an acid phosphatase of *Zymomonas mobilis*) was remarkably similar to that of the ‘pho box’ in *E. coli*. In the best alignment, 12 of 18 bases were conserved in *Zymomonas mobilis phoC*, and five conserved bases in the –10 region were identical [135].

The cleavage of the C-P bond from organophosphonates by phosphonoacetaldehyde hydrolase and C-P lyases is also inducible only under conditions of phosphate limitation [136,137].

According to Kier et al. [138], the production of the PhoN enzyme (class A acid phosphatase) of *Salmonella enterica* serovar *typhimurium* is moderately induced by Pi starvation. However, evidence has also shown that this gene is under the control of the *phoP*-*phoQ* two-component regulatory system [139,140], which promotes transcription of *phoN* and other PhoP activated genes under low environmental Mg^{2+} concentrations [141].

Some *Enterobacteriaceae* species, such as *Morganella morganii* and *Providencia stuartii*, show a peculiar pattern of phosphatase activity consisting of the high-level phosphate-irrepressible production of acid phosphatase activity (HPAP phenotype) [142,143]. *Morganella morganii* produces a major phosphate-irrepressible class A acid phosphatase (named PhoC), which is associated with the HPAP phenotype and a minor phosphate-irrepressible class B acid phosphatase (named NapA). The regulation of the class B enzyme is apparently similar to that of class B phosphatase of *E. coli* [134]. Another example of a Pi irrepressible phosphatase-encoding gene is the *phoD* gene of *Zymomonas mobilis*, which is expressed constitutively [144,145].

These findings indicate that most of alkaline phosphatases found in the family *Enterobacteriaceae* are Pi-repressible, while many of the acid phosphatases are Pi-irrepressible. Other regulatory systems have been proposed for some bacterial phosphatases. In *Pseudomonas fluorescens* MF3, it was determined that the expression of the *apo* gene, which encodes an acidic phosphatase enzyme, was regulated by the growth temperature. The finding of a co-regulation mechanism at the transcriptional level suggests the existence of a new regulatory mechanism for these genes (whose expression is maximal at 17.5°C) as a response to the growth temperature [146]. Furthermore, the *apy* gene of *Shigella flexneri*, encoding an ATP diphosphohydrolase or apyrase, and other related alleles present in virulent *Shigella* spp. and enteroinvasive *E. coli* strains, is expressed in a thermoregulated manner [147].

According to the results of Rossolini et al. [148], in *E. coli* MG1655 the production of the p27 enzyme (acid phosphatase, class B, probably corresponding to the product of the *napA* gene found in this species, [149] appears to be switched off when cells were grown on glucose, and turned on when growth was supported by alternative carbon sources. This behavior suggests that expression of the p27 enzyme is regulated in a complex fashion.

Finally, positive regulation by cyclic adenosine monophosphate (cAMP) and the cAMP receptor protein (CRP) was proposed by Kier et al. [138] for two enzymes produced by *Salmonella typhimurium*, an acid hexose phosphatase and a cyclic phosphodiesterase. This mechanism was proposed by Pradel and Boquet [124,150] for the expression in vivo of the *E. coli agp* gene, which encodes a periplasmic acid glucose-1-phosphate phosphatase. In addition, a negative control by cAMP has been found for the pH 2.5 acid phosphatase gene (appA) from *E. coli* [151].

All of the available evidence indicates that the regulation of phosphatase enzymes is a complex matter that requires considerable additional research. In any event, the existing knowledge about *Enterobacteriaceae* phosphatases constitutes a basis for better understanding and for further exploration of the rules governing phosphatase expression in soil bacteria.

6.1. Isolation and characterization of acid phosphatase encoding genes

The isolation of bacterial phosphatase-encoding genes has been carried out by means of expression cloning systems based on histochemical screening of genomic libraries. These procedures allow quick recognition of clones harboring and expressing the enzymatic activity.

A system based on an indicator medium (named TPMG) containing the phosphatase substrate phenolphthalein diphosphate (PDP) and the stain methyl green (MG) was developed by Riccio et al. [152]. This medium allows identification of the phosphatase positive phenotype (pho$^+$) as green-stained colonies, while the phosphatase negative (pho$^-$) clones grow as unstained colonies. This system has been used for the isolation of several bacterial phosphatase-encoding genes from different species, such as *Providencia sturatii, Providencia rettgeri* and *Morganella morganii* [134,149,152,153].

Another system for expression cloning of bacterial phosphatase-encoding genes is Luria Agar containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP), which permits the direct selection of dark blue transformant colonies on indicator plates. This system was used by Pond et al. [135] for cloning an acid phosphatase-encoding gene (*phoC*) from *Zymomonas mobilis* [154]. Groisman et al. [154] cloned the structural gene for the pH 2.5 acid phosphatase (*appA*) of *E. coli* by a method consisting of an in vivo shotgun cloning technique to amplify directly the genes responsible for high level para-nitrophenyl-phosphate (pNPP) hydrolysis (phosphatase activity). Colonies that stained yellow were considered to be acid phosphatase-positive clones. This technique was also used by Pradel and Boquet [124] to clone the *agp* gene, encoding a periplasmic acid glucose phosphatase of *E. coli.*

By using different expression cloning systems, 14 nonspecific acid phosphatase encoding genes from different bacterial species have been isolated [122]. Sequence analysis of the cloned phosphatase genes and other characteristics has allowed the classification of nonspecific phosphohydrolases into three different families: class A, class B, and class C phosphatases [134,149,153].

High homology at the sequence level has been detected in class A phosphatase genes from *M. morganii* and *P. stuartii*, suggesting that these genes are vertically derived from a common ancestor [122]. The existence of various conserved domains and a signature sequence motif for each family (A, B, and C) of bacterial phosphatases has been confirmed [122].

In addition, several other phosphatase genes have been isolated from *Escherichia coli.* These include: *ushA*, which encodes a 5′-nucleotidase [123]; *agp*, which encodes an acid glucose-1-phosphatase [124,150]; and *cpdB*, encoding the 2′–3′ cyclic phosphodiesterase [155]. A gene from *Providencia stuartii* and *Providencia rettgeri* that encodes the 43-kDa acid-hexose phosphatase [152], as well as a gene cluster involved in the synthesis of specific P-releasing enzyme from organophosphonate substrates (C-P lyase) [30] have also been cloned. These genes may be an interesting source for the further genetic manipulation of soil phosphate-solubilizing bacteria.

7. Future prospects

Phosphate-solubilizing bacteria play an important role in plant nutrition through the increase in P uptake by the plant, and their use as PGPR is an important contribution to biofertilization of agricultural crops. Accordingly, further investigation is needed to improve the performance and use of phosphate-solubilizing bacteria as bacterial inoculants.

Greater attention should be paid to studies and application of new combinations of phosphate-solubilizing bacteria and other PGPR for improved results. The mechanisms explaining the synergistic interaction should be a matter of further research to elucidate the biochemical basis of these interactions. On the other hand, genetic manipulation of phosphate-solubilizing bacteria to improve their phosphate-solubilizing capabilities and/or the introduction of this trait in strains with other plant growth promoting effects is not only important, but also seems to be practically feasible.

In addition, the selection by classical genetic methods of mutants with increased production of organic acids and/or phosphatase activity, could constitute an effective approach that can not be underestimated. Genetic manipulation by recombinant DNA technology seems to offer a feasible approach for obtaining improved strains. Cloning of genes involved in mineral phosphate solubilization, such as those influencing the synthesis of organic acids, as well as phosphatase encoding genes, would be the first step in such a genetic manipulation program. Subcloning of these genes in appropriate vectors and their transfer and expression (or overexpression) in target host strains could be a successful procedure for improving the phosphate solubilization capabilities of selected strains. Recipient strains should be selected either for the expression of a certain phosphate-solubilizing activity, which is to be improved, or for the presence of some other important trait involved in plant growth promotion that would favorably complement the potential to release P from insoluble substrates.

Future research should also investigate the stability and performance of the phosphate solubilization trait once the bacteria have been inoculated in soil, in both natural and genetically modified strains. The survival and establishment of the introduced strain can be affected by low competitiveness, thus limiting the effectiveness of application [156]. On the other hand, the putative risk involved in the release of genetically engineered microorganisms in soil is a matter of controversy, in particular with regard to the possibility of horizontal transfer of the inserted DNA to other soil microorganisms [157]. For these reasons, the use of genetic reporter systems, such as bioluminescence genes [158,159], or green fluorescent protein genes [160] is crucial in studying the fate and survival of the strain in soil.

Genetic engineering of the phosphate solubilizing character must eventually be directed to the chromosomal integration of the gene for higher stability of the character and to avoid horizontal transfer of the inserted gene in soil. This strategy would also prevent the risk of metabolic load caused by the presence of the plasmid in the bacterial cell [161]. On the other hand, chromosomal integration may have the disadvantage of a low expression of the activity, due to the low copy number of the gene, in comparison with plasmid-harbored genes. An alternative to this situation might be the integration of multicopies of the target gene. Additionally, the use of powerful and species-specific promoters, which could be activated under the specific environmental conditions of soil is another interesting approach to successful gene expression in the engineered strain.

References

[1] Suslov TV. Role of root-colonizing bacteria in plant growth. In: Mount MS, Lacy GH, editors. Phytopathogenic Prokariotes. London: Academic Press, 1982. pp. 187–223.

[2] Davinson J. Plant beneficial bacteria. Bio/Technology 1988;6:282–6.

[3] Lemanceau P. Effects benefiques de rhizobacteries sur les plantes: exemple des *Pseudomonas* spp. fluorescent. Agronomie 1992;12:413–37.

[4] Kloepper JW. Plant growth promoting bacteria (other systems). In: Okon J, editor. Azospirillum/Plant Association. Boca Raton, FL: CRC Press, 1994. pp. 137–54.

[5] Glick BR. The enhancement of plant growth by free-living bacteria. Can J Microbiol 1995a:41:109–17.
[6] Kloepper JW, Schroth MN. Plant growth-promoting rhizobacteria on radishes. In: Station de Pathologie vegetale et Phyto-bacteriologie, editor. Proceedings of the 4th International Conference on Plant Pathogenic Bacteria Vol II. Tours: Gilbert-Clary, 1978. pp. 879–82.
[7] Hall JA, Pierson D, Ghosh S, Glick BR. Root elongation in various agronomic crops by the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. Isr J Plant Sci 1996;44:37–42.
[8] Glick BR, Changping L, Sibdas G, Dumbroff EB. Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. Soil Biol Biochem 1997;29:1233–9.
[9] Kloepper JW, Lifshitz K, Schroth MN. *Pseudomonas* inoculants to benefit plant production. ISI Atlas Sci Anim Plant Sci 1988. pp. 60–4.
[10] Kloepper JW, Lifshitz K, Zablotowicz RM. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 1989;7:39–43.
[11] Brown ME. Seed and root bacterization. Annu Rev Phytopatol 1974;12:181–97.
[12] Kapulnik J, Gafny R, Okon Y. Effect of *Azopirillum* spp. inoculation on root development and NO-3 uptake in wheat (*Titicum aestivum* cv. Miriam) in hydroponic systems. Can J Bot 1985;63:627–31.
[13] Baldani VLD, Baldani JI, Döbereiner J. Inoculation on field-grown wheat (*Triticum aestivum*) with *Azospirillum* spp. in Brazil. Biol Fert Soils 1987;4:37–40.
[14] Sarig S, Okon Y, Blum A. Promotion of leaf area development and field in *Sorghum bicolor* inoculated with *Azospirillum brasilense.* Symbiosis 1990;9:235–45.
[15] Broadbent P, Baker KF, Franks N, Holland J. Effect of *Bacillus* spp. on incrased growth of seedlings in steamed and in nontreated soil. Phytopathology 1977;67:1027–34.
[16] Burr TJ, Schroth MN, Suslow T. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *Pseudomonas putida.* Phytopathology 1978;68:1377–83.
[17] Capper AL, Campbell R. The effect of artificially inoculated antagonistic bacteria on the prevalence of take-all disease of wheat in field experiment. J Appl Bacteriol 1986;60:155–60.
[18] Sivan A, Chet I. Microbial control of plant diseases. In: Mitchell R, editor. Environmental Microbiology. New York: Wiley-Liss, 1992. pp. 335–54.
[19] Leong J. Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. Annu Rev Phytopathol 1986;24:187–208.
[20] Xie H, Pasternak JJ, Glick BR. Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indoleacetic acid. Curr Microbiol 1996;32:67–71.
[21] Christiansen-Weneger C. N2-fixation by ammonium-excreting *Azospirillum brasilense* in auxin-induced tumours of wheat (*Triticum aestivum* L.). Biol Fertil Soils 1992;12:85–100.
[22] Bashan Y, Levanony H. Alterations in membrane potential and in proton efflux in plant roots induced by *Azospirillum brasilense.* Plant Soil 1991;137:99–103.
[23] Glick BR, Penrose DM, Li J. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 1998;190:63–8.
[24] Krasilnikov M. On the role of soil bacteria in plant nutrition. J Gen Appl Microbiol 1961;7:128–44.
[25] Gaur AC, Ostwal KP. Influence of phosphate dissolving Bacilli on yield and phosphate uptake of wheat crop. Indian J Exp Biol 1972;10:393–4.
[26] Subba Rao NS. Advances in agricultural microbiology. In: Subba Rao NS, editor. Studies in the Agricultura and Food Sciences. London: Butterworth Scientific, 1982. pp. 295–303.
[27] Ehrlich HL. Mikrobiologische und biochemische Verfahrenstechnik. In: Einsele A, Finn RK, Samhaber W, editors. Geomicrobiology, 2nd ed. Weinheim: VCH Verlagsgesellschaft, 1990.
[28] Fernández C, Novo R. Vida Microbiana en el Suelo, II. La Habana: Editorial Pueblo y Educación, 1988.
[29] Begon M, Harper JL, Townsend CR. Ecology: Individuals, Populations and Communities, 2nd ed. Blackwell Scientific Publications USA, 1990.
[30] Ohtake H, Wu H, Imazu K, Ambe Y, Kato J, Kuroda A. Bacterial phosphonate degradation, phosphite oxidation and polyphosphate accumulation. A Res Conserv and Recycling 1996;18:125–34.
[31] Goldstein AH. Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram-negative bacteria. In: Torriani-Gorini A, Yagil E, Silver, S, editors. Phosphate in Microorganisms: Cellular and Molecular Biology. Washington, DC: ASM Press, 1994. pp. 197–203.

[32] Beever RE, Burns DJW. Phosphorus uptake, storage and utilization by fungi. Adv Bot Res 1980;8:127–219.
[33] Odum EP. Fundamentos de Ecologìa. Mexico: Interamericana, 1986.
[34] Roychoudhury P, Kaushik BD. Solubilization of Mussorie rock phosphate by cyanobacteria. Curr Sci 1989;58:569–70.
[35] Halder AK, Mishra AK, Bhattacharyya P, Chakrabartty PK. Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium.* J Gen Appl Microbiol 1990;36:81–92.
[36] Richardson AE. Soil microorganisms and phosphorus availability. In: Pankhurst CE, Doube BM, Grupta VVSR, Grace PR, editors. Soil Biota, Management in Sustainable Farming Systems. Melbourne, Australia: CSIRO, 1994, pp. 50–62.
[37] Dey KB. Phosphate solubilizing organisms in improving fertility status. In: Sen SP, Palit P, editors. Biofertilizers: Potentialities and Problems. Calcutta: Plant Physiology Forum, Naya Prokash,1988. pp. 237–48.
[38] Goldstein AH. Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am J Altern Agri 1986;1:51–7.
[39] Jones DA, Smith BFL, Wilson MJ, Goodman BA. Solubilizator fungi of phosphate in rise soil. Mycol Res 1991;95:1090–3.
[40] Lindsay WL. Chemical Equilibrial in Soil. New York: John Wiley and Sons, 1979.
[41] Paul EA, Clark FE. Soil Microbiology and Biochemistry. San Diego, CA: Academic Press, 1988.
[42] Dalal RC. Soil organic phosphorus. Adv Agron 1977;29:83–117.
[43] Anderson G. Assessing organic phosphorus in soils. In: Khasawneh FE, Sample EC, Kamprath EJ, editors. The Role of Phosphorus in Agriculture. Madison, Wis: Amer Soc Agronomy, 1980. pp. 411–32.
[44] Harley JL, Smith SE. Mycorrhizal symbiosis. London, New York: Academic Press, 1983.
[45] McGrath JW, Wisdom GB, McMullan G, Lrakin MJ, Quinn JP. The purification and properties of phosphonoacetate hydrolase, a novel carbon-phosphorus bond-cleaving enzyme from *Pseudomonas fluorescens* 23F. Eur J Biochem 1995;234:225–30.
[46] McGrath JW, Hammerschmidt F, Quinn JP. Biodegradation of phosphonomycin by *Rhizobium huakuii* PMY1. Appl Environ Microbiol 1998;64:356–58.
[47] Sperberg JI. The incidence of apatite-solubilizing organisms in the rhizosphere and soil. Aust J Agric Res 1958;9:778.
[48] Katznelson H, Peterson EA, Rovatt JW. Phosphate dissolving microoganisms on seed and in the root zone of plants. Can J Bot 1962;40:1181–6.
[49] Raghu K, MacRae IC. Occurrence of phosphate-dissolving microorganisms in the rhizosphere of rice plants and in submerged soils. J Appl Bacteriol 196629:582–6.
[50] Alexander M. Introduction to Soil Microbiology. New York: Wiley and Sons, 1977.
[51] Louw HA, Webley DM. A study of soil bacteria dissolving certain phosphate fertilizers and related compounds. J Appl Bacteriol 1959;22:227–33.
[52] Das AC. Utilization of insoluble phosphates by soil fungi. J Indian Soc Soil Sci 1963;11:203–7.
[53] Ostwal KP, Bhide VP. Solubilization of tricalcicum phosphate by soil *Pseudomonas.* Indian J Exp Biol 1972;10:153–4.
[54] Bardiya MC, Gaur AC. Isolation and screening of microorganisms dissolving low grade rock phosphate. Folia Microbiol 1974;19:386–9.
[55] Darmwall NS**,** Singh RB, Rai R. Isolation of phosphate solubilizers from different sources. Curr Sci 1989;58:570–1.
[56] Illmer P, Schinner F. Solubilization of inorganic phosphates by microorganisms isolated from forest soil. Soil Biol Biochem 1992;24:389–95.
[57] Goldstein AH, Liu ST. Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola.* Bio/Technology 1987;5:72–4.
[58] Gupta R, Singal R, Sankar A, Chander RM, Kumar RS. A modified plate assay for screening phosphate solubilizing microorganisms. J Gen Appl Microbiol 1994;40:255–60.
[59] Babenko YS, Tyrygina G, Grigoryev EF, Dolgikh LM, Borisova TI. Biological activity and physiologo-biochemical properties of bacteria dissolving phosphates. Microbiologiya 1984;53:533–9.
[60] Khan JA, Bhatnagar RM. Studies on solubilization of insoluble phosphate rocks by *Aspergillus niger* and *Penicillium sp.* Fertil Technol 1977;14:329–33.

[61] Rodríguez H, Goire I, Rodríguez M. Caracterización de cepas de *Pseudomonas* solubilizadoras de fósforo. Rev ICIDCA 1996;30:47–54.
[62] Cabala-Rosand P, Wild A. Direct use of low grade phosphate rock from Brazil as fertilizer II. Effects of mycorrhiza inoculation and nitrogen source. Plant Soil 1982;65:363–73.
[63] Kucey RMN. Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can J Soil Sci 1983;63:671–8.
[64] Arora D, Gaur C. Microbial solubilization of different inorganic phosphates. Indian J Exp Biol 1979;17: 1258–61.
[65] Lynch JM. Microbial metabolites. In: Lynch JM, editor. The Rhizosphere. Chichester, England: John Wiley and Sons Ltd, Baffins Lane, Interscience, 1990. pp. 177–206.
[66] El-Sawah MMA, Hauka FIA, El-Rafey HH. Study on some enzymes cleaving phosphorus from organic substrates in soil. J Agric Sci 1993;18:2775–85.
[67] Bishop ML, Chang AC, Lee RWK. Enzymatic mineralization of organic phosphorus in a volcanic soil in Chile. Soil Sci 1994;157:238–43.
[68] Feller C, Frossard E, Brossard M. Phosphatase activity in low activity tropical clay soils. Distribution in the various particle size fractions. Can J Soil Sci 1994;74:121–9.
[69] Kremer RJ. Determination of soil phosphatase activity using a microplate method. Comun Soil Sci Plant Anal 1994;25:319–25.
[70] Sarapatka B, Kraskova M. Interactions between phosphatase activity and soil characteristics from some locations in the Czech Republic. Rostlinna-Vyroba-UZPI 1997;43:415–9.
[71] Kirchner MJ, Wollum AG, King LD. Soil microbial populations and activities in reduced chemical input agroecosystems. Soil Sci Soc Amer J 1993;57:1289–95.
[72] Kucharski J, Ciecko Z, Niewolak T, Niklewska-Larska T. Activity of microrganisms in soils of different agricultural usefulness complexes fertilized with mineral nitrogen. Acta Acad Agric Technicae-Olstenensis 1996;62:25–35.
[73] Garcia C, Fernandez T, Costa F, Cerranti B, Masciandaro G. Kinetics of phosphatase activity in organic wastes. Soil Biol Biochem 1992;25:361–5.
[74] Xu JG, Johnson RL. Root growth, microbial activity and phosphatase activity in oil-contaminated, remediated and uncontaminated soils planted to barley and field pea. Plant Soil 1995;173:3–10.
[75] Tarafdar JC, Junk A. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol Fertil Soil 1987;3:199–204.
[76] Greaves MP, Webley DM. A study of the breakdown of organic phosphates by microorganisms from the root region of certain pasture grasses. J Appl Bact 1965;28:454–65.
[77] Abd-Alla MH. Use of organic phosphorus by *Rhizobium leguminosarum biovar. viceae* phosphatases. Biol Fertil Soils 1994;18:216–8.
[78] Fox TR, Comerford NB. Rhizosphere phosphatase activity and phosphatase hydrolysable organic phosphorus in two forested spodosols. Soil Biol Biochem 1992;24:579–83.
[79] Burns RG. Extracellular enzyme-substrate interactions in soil. In: Slater JH, Whittenbury R, Wimpenny JWT, editors. Microbes in their Natural Environment. Cambridge: Cambridge Univ Press, 1983. pp. 249–98.
[80] Thaller MC, Berlutti F, Schippa S, Iori P, Passariello C, Rossolini GM. Heterogeneous patterns of acid phosphatases containing low-molecular-mass Polipeptides in members of the family *Enterobacteriaceae.* Int J Syst Bacteriol 1995b;4:255–61.
[81] Gügi B, Orange N, Hellio F, Burini JF, Guillou C, Leriche F, Guespin-Michel JF. Effect of growth temperature on several exported enzyme activities in the psychrotropic bacterium *Pseudomonas fluorescens.* J Bacteriol 1991;173:3814–20.
[82] Skrary FA, Cameron DC. Purification and characterization of a *Bacillus licheniformis* phosphatase specific for D-alpha-glycerphosphate. Arch Biochem Biophys 1998;349:27–35.
[83] Richardson AE, Hadobas PA. Soil isolates of *Pseudomonas* spp. that utilize inositol phosphates. Can J Microbiol 1997;43:509–16.
[84] Tarafdar JC, Claassen N. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol Fertil Soils 1988:5:308–12.
[85] Gerretsen FC. The influence of microorganisms on the phosphate intake by the plant. Plant Soil 1948:1:51–81.

[86] Cooper R. Bacterial fertilizers in the Soviet Union. Soils Fertilizers 1959;22:327–30.
[87] Datta M, Banish S, Dupta RK. Studies on the efficacy of a phytohormone producing phosphate solubilizing *Bacillus firmus* in augmenting paddy yield in acid soils of Nagaland. Plant Soil 1982;69:365–73.
[88] Kucey RMN, Janzen HH, Leggett ME. Microbially mediated increases in plant-available phosphorus. Adv Agron 1989;42:199–228.
[89] Lifshitz R, Kloepper JW, Kozlowski M, Simonson C, Carlson J, Tipping EM, Zalesca I. Growth promotion of canola (rapeseed) seedlings by a strain of *Psedomonas putida* under gnotobiotic conditions. Can J Microbiol 1987;33:390–5.
[90] Chabot R, Antoun H, Cescas MP. Stimulation de la croissance du mais et de la laitue romaine par desmicroorganismes dissolvant le phosphore inorganique. Can J Microbiol 1993;39:941–7.
[91] Chabot R, Antoun H, Kloepper JW, Beauchamp CJ. Root colonization of maize and lettuce by bioluminiscent *Rhizobium leguminosarum* biovar. *phaseoli.* Appl Environ Microbiol 1996a;62:2767–72.
[92] Chabot R, Hani A, Cescas PM. Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar. *phaseoli.* Plant Soil 1996b;184:311–21.
[93] Murty MG, Ladha JK. Influence of *Azospirillum* inoculation on the mineral uptake and growth of rice under hydroponic conditions. Plant Soil 1988;108:281–5.
[94] Fernández HM, Carpena AO, Cadakia LC. Evaluacion de la solubilizacion del fósforo mineral en suelos calizos por *Bacillus cereus.* Ensayos de invernadero. Anal Edaf Agrobiol 1984;43:235–45.
[95] Kundu BS, Gaur AC. Rice responce to inoculation with N2-fixing and P-soluvilizing microorganisms. Plant Soil 1984;79:227–34.
[96] Monib M, Hosny I, Besada YB. Seed inoculation of castor oil plant (*Ricinus communis*) and effect on nutrient uptake. Soil Biol Conserv Biosphere 1984;2:723–32.
[97] Alagawadi AR, Gaur AC. Inoculation of *Azospirillum brasilense* and phosphate-solubilizing bacteria on yield of sorghum [*Sorghum bicolor*(L.) Moench] in dry land. Trop Agric 1992;69:347–50.
[98] Belimov AA, Kojemiakov AP, Chuvarliyeva CV. Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant Soil 1995;173:29–37.
[99] Garbaye J. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 1994;128:197–210.
[100] Frey-Klett P, Pierrat JC, Garbaye J. Location and survival of mycorrhiza helper *Pseudomonas fluorescens* during establishment of ectomycorrhizal symbiosis between *Laccaria bicolor* and Douglas fir. Appl Environ Microbiol 1997:63;139–44.
[101] Ray J, Bagyaraj DJ, Manjunath A. Influence of soil inoculation with versicular arbuscular mycorrhizal (VAM) and a phosphate dissolving bacteria on plant growth and 32P uptake. Soil Biol Biochem 1981;13:105–8.
[102] Azcón-Aguilar C, Gianinazzi-Pearson V, Fardeau JC, Gianinazzi S. Effect of vesicular-arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria on growth and nutrition of soybean in a neutral-calcareus soil amended with 32P-45Ca-tricalcium phosphate. Plant Soil 1986;96:3–15.
[103] Piccini D, Azcón R. Effect of phosphate-solubilizing bacteria and versicular arbuscular mycorrhizal (VAM) on the utilization of bayoran rock phosphate by alfalfa plants using a Sand-vermiculite medium. Plant Soil 1987;101:45–50.
[104] Jeffries P, Barea JM. Bioeochemical cycling and arbuscular mycorrhizas in the sustainability of plant-soil system. In: Gianinazzi S, Schüepp H, editors. Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. Basel, Switzerland: Birkhäuser Verlag, 1994. pp. 101–15.
[105] Toro M, Azcón R, Barea JM. Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (32P) and nutrient cycling. Appl Environ Microbiol 1997;63:4408–12.
[106] Smith JH, Allison FE, Soulides DA. Phosphobacteria as a soil inoculant. Tech US Dept Agricult Bul 1962;1:63–70.
[107] Duff RB, Webley DM. 2-Ketogluconic acid as a natural chelator produced by soil bacteria. Chem Ind 1959;1376–77.
[108] Sundara Rao WVB, Sinha MK. Phosphate dissolving micro-organisms in the soil and rhizosphere. Indian J Agric Sci 1963;33:272–8.
[109] Banik S, Dey BK. Available phosphate content of an alluvial soil is influenced by inoculation of some isolated phosphate-solubilizing microorganisms. Plant Soil 1982;69:353–64.

[110] Craven PA, Hayasaka SS. Inorganic phosphate solubilization by rhizosphere bacteria in a *Zostera marina* community. Can J Microbiol 1982;28:605–10.

[111] Leyval C, Berthelin J. Interaction between *Laccaria laccata*, *Agrobacterium radiobacter* and beech roots: influence on P, K, Mg and Fe movilization from minerals and plant growth. Plant Soil 1989;117:103–10.

[112] Salih HM, Yahya AY, Abdul-Rahem AM, Munam BH. Availability of phosphorus in a calcareus soil treated with rock phosphate or superphosphate as affected by phosphate dissolving fungi. Plant Soil 1989;120:181–5.

[113] Liu TS, Lee LY, Tai CY, Hung CH, Chang YS, Wolfram JH, Rogers R, Goldstein AH. Cloning of an *Erwinia herbicola* gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in *Escherichia coli HB101*: Nucleotide sequence and probable involvement in biosynthesis of the coenzyme pyrroloquinoline quinone. J Bacteriol 1992;174:5814–9.

[114] Goldstein AH, Rogers RD, Mead G. Mining by microbe. Bio/Technology 1993;11:1250–4.

[115] Halder AK, Chakrabartty PK. Solubilization of inorganic phosphate by *Rhizobium.* Folia Microbiol 1993;38:325–30.

[116] Goldstein AH. Recent progress in understanding the molecular genetics and biochemestry of calcium phosphate solubilization by gram negative bacteria. Biol Agric Hortic 1995;12:185–93.

[117] Thomas GV. Occurrence and ability of phosphate-solubilizing fungi from coconut plant soils. Plant Soil 1985;87:357–64.

[118] Asea PEA, Kucey RMN, Stewart JWB. Inorganic phosphate solubilization by two *Penicillium* sp. in solution culture and soil. Soil Biol Biochem 1988;20:459–64.

[119] Rudolfs W. Influence of sulfur oxidation upon growth of soy beans and its effect on bacterial flora of soil. Soil Sci 1922;14:247–62.

[120] Hopkins CG, Whiting AL. Soil bacteria and phosphates. III. Agric Exp Stn Bull 1916;190:395–406.

[121] Vázquez P. México. Bacterias solubilizadoras de fosfatos inorgánicos asociadas a la rhizosfera de los mangles: *Avicennia germinans* (L.) L y *Laguncularia racemosa* (L.) Gerth. Tesis para el título de Biologo Marino. Univ. Autónoma de Baja California Sur. La Paz, B.C.S. 1996.

[122] Rossolini GM, Shippa S, Riccio ML, Berlutti F, Macaskie LE, Thaller MC. Bacterial nonspecific acid phosphatases: physiology, evolution, and use as tools in microbial biotechnology. Cell Mol Life Sci 1998;54:833–50.

[123] Burns DM, Beacham IR. Nucleotide sequence and transcriptional analysis of the *Escherichia coli ushA* gene, encoding periplasmic UDP-sugar hydrolase (5′-nucleotidase): regulation of the ushA gene, and the signal sequence of its encoded protein product. Nucleic Acids Res 1986;14:4325–42.

[124] Pradel E, Boquet PL. Acid phosphatases of *Escherichia coli*: molecular cloning and analysis of *agp*, the structural gene for a periplasmic acid glucose phosphatase. J Bacteriol 1988;170:4916–23.

[125] Cosgrove DJ, Irving GCJ, Bromfield SM. Inositol phosphate phosphatases of microbial origin. The isolation of soil bacteria having inositol phosphate phosphatase activity. Aust J Biol Sci 1970;23:339–43.

[126] Bujacz B, Wieczorek P, Krzysko-Lupcka T, Golab Z, Lejczak B, Kavfarski P. Organophosphonate utilization by the wild-type strain of *Penicillium notatum.* Appl Environ Microbiol 1995;61:2905–10.

[127] Babu-Khan S, Yeo TC, Martin WL, Duron MR, Rogers RD, and Goldstein AH. Cloning of a mineral phosphate-solubilizing gene from *Pseudomonas cepacia.* Appl Environ Microbiol 1995;61:972–8.

[128] Goosen N, Horsman HP, Huinen RG, van de Putte P. *Acinetobacter calcoaceticus* genes involved in biosynthesis of the coenzyme pyrrolo-quinoline-quinone: nucleotide sequence and expression in *Escherichia coli* K-12. J Bacteriol 1989;171:447–55.

[129] van Schie BJ, Hellingwerf KJ, van Dijken JP, Elferink MGL, van Dijl JM, Kuenen JG, Konigns WN. Energy transduction by electron transfer via a pyrrolo-quinoline quinone-dependent glucose dehydrogenase in *Escherichia coli*, *Pseudomonas putida*, and *Acinetobacter calcoaceticus* (var. *lwoffii*). J Bacteriol 1987;163:493–9.

[130] Mikanova O, Kubat J, Simon T, Vorisek K, Randova D. Influence of soluble phosphate on P-solubilizing activity of bacteria. Rostlinna-Vyroba-UZPI 1997;43:421–4.

[131] Wanner BL. Cellular and molecular biology phosphate regulation of gene expression in *E. coli*. In: Niedhardt FC, Ingraham JL, Low KB, Magasanik B, Schaechter M, Umbarger HE, editors. *Escherichia coli* and *Salmonella typhimurium*. Washington, DC: ASM Press, 1987. pp. 1326–33.

[132] Rosenberg H. Phosphate transport in prokariotes. In: Rosen B, Silver S, editors. Ion Transport in Prokaryotes. San Diego, CA: Academic Press, Inc, 1987. pp. 205–48.

[133] Torriani-Gorini A. Regulation of phosphate metabolism and transport. In: Torriani-Gorini A, Yagil E, Silver S, editors. Phosphate in Microorganisms: Cellular and Molecular Biology. Washington, DC: ASM Press, 1994. pp. 1–4.

[134] Thaller MC, Berlutti F, Schippa S, Lombardi G, Rossolini GM. Characterization and sequence of PhoC, the principal phosphate-irrepressible acid phosphatase of *Morganella morganii.* Microbiology 1994;140:1341–50.

[135] Pond JL, Eddy CK, Mackenzie KF, Conway T, Borecky DJ, Ingram LO. Cloning, sequencing and characterization of the principal acid phosphatase, phoC product, from *Zymomonas mobilis.* J Bacteriol 1989;171:767–74.

[136] Wackett LP, Shames SL, Venditti CP, Walsh CT. Bacterial carbon-phosphorus lyase: products, rates and regulation of phosphonic and phosphonic acid metabolism. J Bacteriol 1987;169:710–7.

[137] Kertesz M, Elgorriaga A, Amrhein N. Evidence for two distinct phosphonate degrading enzymes (C-P lyases) in *Arthrobacter* sp. GLP-1. Biodegradation 1991;2:53–9.

[138] Kier LD, Weppelman R, Ames BN. Resolution and purification of three phosphatases of *Salmonella typhimurium.* J Bacteriol 1977;130:411–9.

[139] Kier LD, Weppelman R, Ames BN. Regulation of nonspecific acid phosphatase in Salmonella: *phoN* and *phoP* genes. J Bacteriol 1979;138:155–61.

[140] Miller SI, Kukral AM, Mekalanos JJ. A two-component regulatory system (*phoP-phoQ*) controls *Salmonella typhimurium* virulence. Proc Natl Acad Sci USA 1989;86:5054–8.

[141] Vescovi EG, Soncini FC, Groisman EA. Mg2+ as an extracellular signal: environmental regulation of *Salmonella* virulence. Cell 1996;84:165–74.

[142] Pompei R, Cornagli G, Ingianni A, Satta G. Use of a novel phosphatase test for simplified identification of species of the tribe proteae. J Clin Microbiol 1990;28:1214–8.

[143] Pompei R, Ingianni A, Foddis G, Di Pietro G, Satta G. Patterns of phosphatase activity among enterobacterial species. Int J Syst Bacteriol 1993;43:174–8.

[144] Reyes L, Scopes RK. The use of multifunctional adsorbents to purify membrane-bound phosphatases from *Zymomonas mobilis.* Bioseparation 1991;2:137–46.

[145] Baoudene-Aassali F, Baratti J, Michel GPF. Purification and properties of a phosphate irrepressible membrane-bound alkaline phosphatase from *Zymomonas mobilis.* J Gen Microbiol 1993;139:229–35.

[146] Burini JF, Gügi B, Merieau A, Janine FGM. Lipase and acidic phosphatase from the psychotrophic bacterium *Pseudomonas fluorescens*: Two enzymes whose synthesis is regulated by the growth temperature. FEMS Microbiol Lett 1994;122:13–8.

[147] Bhargava T, Datta S, Ramachandran V, Ramakrishnan R, Roy RK, Sankaran K, Subrahmanyam YVBK. Virulent *Shigella* codes for a soluble apyrase: identification, characterization and cloning of the gene. Curr Sci 1995;68:293–300.

[148] Rossolini GM, Thaller MC, Pezzi R, Satta G. Identification of an *Escherichia coli* periplasmic acid phosphatase containing a 27 kDa-polypeptide component. FEMS Microbiol Lett 1994;118:167–74.

[149] Thaller MC, Giovanna L, Serena S, Rossolini GM. Cloning and characterization of the NapA acid phosphatase phosphotransferase of *Morganella morganii*: identification of a new family of bacterial acid-phosphatase-encoding genes. Microbiology 1995a;141:147–54.

[150] Pradel E, Boquet PL. Nucleotide sequence and transcriptional analysis of the *Escherichia coli agp* gene encoding periplasmic acid glucose-1-phosphatase. J Bacteriol 1990;172:802–7.

[151] Touati E, Danchin A. The structure of the promoter and amino terminal region of the pH 2.5 acid phosphatase gene (appA) of *E. coli*: a negative control of transcription mediated by cyclic AMP. Biochimie 1987;69:215–21.

[152] Riccio ML, Rossolini GM, Lombardi G, Chiesurin A, Satta G. Expression cloning of different bacterial phosphatase-encoding genes by histochemical screening of genomic libraries onto an indicator medium containing phenolphthalein diphosphate and metyl green. J Appl Bacteriol 1997;82:177–85.

[153] Thaller MC, Schippa S, Bonci A, Cresti S, Rossolini GM. Identification of the gene (*aphA*) encoding the class B acid phosphatase/phosphotransferase of *Escherichia coli* MG 1655 and characterization of its product. FEMS Microbiol Lett 1997;146:191–8.

[154] Groisman EA, Castillo BA, Casadaban MJ. In vivo DNA cloning and adjacent gene fusing with a mini-Mu-lac bacteriophage containing a plasmid replicon. Proc Natl Acad Sci USA 1984;81:1480–3.

[155] Beacham IR, Garrett S. Isolation of *Escherichia coli* mutants (*cpdB*) deficient in periplasmic 2′:3′-cyclic phosphodiesterase and genetic mapping of the *cpdB* locus. J Gen Microbiol 1980;119:31–4.

[156] van Elsas JD, Dijkstra AF, Govarert JM, van Veen JA. Survival of *Pseudomonas fluorescens* and *Bacillus subtilis* introduced into soils of different texture in field microplots. FEMS Microbiol Ecol 1986;38:150–60.

[157] van Elsas JD, Hekman W, van Overbeek LS, Smith E. Problems and perspectives of the application of genetically engineered microorganisms to soil. Trends Soil Sci 1991;1:373–92.

[158] Shaw JJ, Dane F, Geiger D, Kloepper JW. Use of bioluminescence for detection of genetically engineered microorganims released into the environment. Appl Environ Microbiol 1992;58:267–73.

[159] Flemming CA, Lee H, Trevors JT. Bioluminescent most-probable-number-method to enumerate lux-marked *Pseudomonas aeruginosa* UG2 Lr in soil. Appl Environ Microbiol 1994;60:3458–61.

[160] Andersen JB, Sternberg C, Poulsen LK, Bjorn SP, Giuskov M, Molin S. New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. Appl Environ Microbiol 1998;64:2240–6.

[161] Glick BR. Metabolic load and heterologous gene expression. Biotechnol Adv 1995b;13:247–61.