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Mineral Phosphate Solubilization: Agronomic Implications,Mechanism and Molecular Genetics

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Phosphorus is an essential nutrient required by the plant for vital cellular functions. However, its availability in the rhizosphere is highly limited due to chemical reactions that fix it into insoluble forms. Alternatively plenty of less reactive rock phosphate ores are available in India and needs to be exploited. The soil naturally has bacteria capable of bioameliorating the soil Pi by converting the insoluble forms into soluble orthophosphates that can be taken up by the plant. Such phenomenon has been referred to as mineral phosphate solubilization (MPS). Addition of bacteria capable of MPS activity has been documented to increase plant growth and yield. The mechanism of mineral phosphate solubilization by bacteria has been postulated to be due to release of organic acid and/or due to proton extrusion. Soil inoculation with rock phosphate plus MPS organisms resulted in enhanced plant growth and yield. These studies were carried out mostly with MPS fungi and Gram-positive bacteria. More recently Gram-negative bacteria have been investigated for MPS activity. The recent studies on genetics and molecular biology of MPS activity using Gram-negative bacteria brought out the repression of this activity by external supplementation of Pi indicating the existence of regulatory controls common to *Pho* regulon and *mps* genes

Key Words: Mineral Phosphate Solubilization, Agronomic Implications, Mechanism and Molecular Genetics

Introduction

Phosphorus is one of the essential nutrients for plant growth. It is an integral part of the cellular activities of living organisms. It has a defined role in plant metabolisms such as cell division, development, photosynthesis, breakdown of sugar, nutrient transport within the plant, transfer of genetic characteristics from one generation to another and regulation of metabolic pathways (Tandon 1987, Armstrong 1988, Theodorou & Plaxton 1993). Phosphorus is a frequently limiting macronutrient next only to nitrogen for plant growth and makes up about 0.2% of plant dry weight (Schachtman et al. 1998). The plants obtain their P requirements from the soil pool. It occurs in soils as inorganic phosphate, produced by weathering of parent rock or as organic phosphate derived from decayed plant, animal or microorganisms.

Considerable interest in the past has gone into the development of rock phosphate directly as fertilizer, mainly because nearly 60% of the 145 million tonnes of rock phosphate deposits in India are considered to be very inefficient ore for

manufacture of phosphate fertilizer through wet processing (Jaggi 1981, 1986). Mussorie Rock Phosphate (MRP), the most reactive of different rock phosphates available in India has been used in acid soils (Tandon 1987). The mineral phosphate solubilizing microbes, which acidify the substrate and possibly the rhizosphere, have been used in unison with rock phosphate so that the phosphorus in the rock phosphate can be made available in the soil for plant uptake (Jisha & Alagawadi 1996). Development of superior MPS strains for use along with rock phosphate should be given immense importance. A package of inoculant containing materials that increase the release of Pi from rock phosphate should include the MPS bacteria. The use of such approach would reduce the pollution caused by excess application of phosphate fertilizer. Additionally, the by-products of the reaction of phosphate ore with sulphuric acid at high temperatures are potential water and air pollutants.

This review focuses on the soil P, its uptake by plants, relative unavailability and how the metabolic diversity of bacteria, especially Gram-negative bacteria allows for bioamelioration of Pi in the soil. It also focuses attention on the lean areas of research, particularly genetics and molecular biology, mineral phosphate solubility (MPS) and how it has been studied in the recent past.

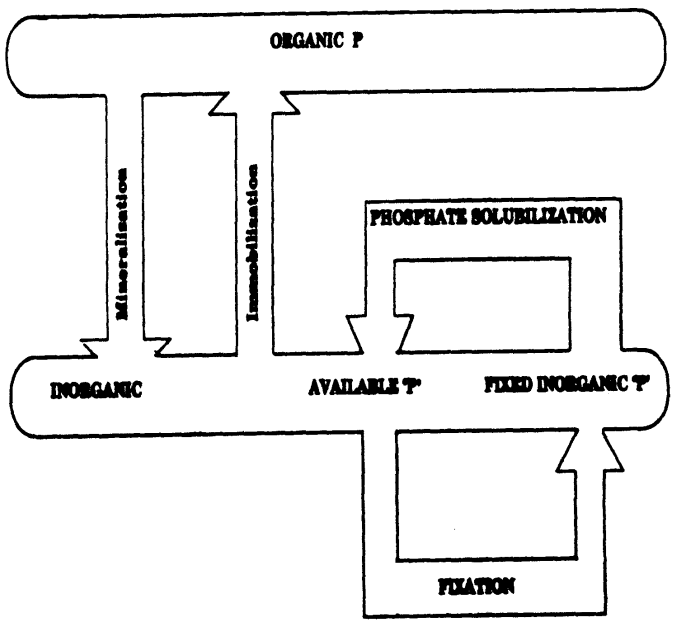

Figure 1 Phosphorus channel in soil

Phosphorus in Soil

Soil is a dynamic system and is an ecological niche of constant biological activity, influenced to a great extent by the plant and the chemical nature of its parent material and the plant growth it supports. The Pi available for biosynthetic purposes will depend not only on the total amount of phosphorus in the environment but also on its solubility, which in turn is dictated by several chemical reactions and biological interaction in the soil. The plants have to pick up their Pi requirements from soil and the soil bacteria too must accumulate Pi from the soil for their biochemical activities. Recycling of phosphate in the soil is depicted in figure 1. The diverse soil P forms can be generally categorized as soil solution P, insoluble organic and insoluble inorganic P. In any agriculture system, not all the applied phosphorus is available to the crop.

In soil, the two reactions, fixation and immobilization convert applied phosphorus into forms unavailable for the plant. More than 70-90% of the applied phosphatic fertilizers get fixed in the soil rendering them unavailable for plant uptake (Bagyaraj & Varma 1995, Larsen 1967, Kardekar & Talashikar 1977, Stevenson 1986, Holford 1997). The decomposing roots also release phosphorus as a result of autolysis, directly into the soil solution mainly as inorganic orthophosphates. The ultimate result of this also is fixation (Martin & Cunningham 1973). Great percentages of soil P is also converted to organic forms of which inositol hexaphosphate is usually a major component, and thus get immobilized and not available for plant growth (Richardson 1994). Central to this review is the concept of conversion of inorganic unavailable phosphate into available forms viz., $H_2PO_4^-$ and HPO_4^{-2} for plant uptake, a phenomenon referred to as mineral phosphate solubilization (MPS). The form in which Pi exists also changes according to the soil *p*H. Below *p*H 6.0, most Pi will be present as monovalent H_2PO_4 species. The plant uptake is also high at the *p*H range of 5.0 – 6.0, which indicates that P is primarily taken up as monovalent form (Furihata et al. 1992). The average orthophosphate concentration in the soil solution of around 10^{-6} M is near the limit at which plants can absorb adequate phosphate. However, critical (threshold) concentrations vary for different plant

species. Phosphorus enters the plant root hairs, root tips and the outer most layers of the root cells. Once inside the root, the inorganic P is stored in the root or transported into the upper portions of the plant. There through various chemical reactions it is incorporated into organic compounds.

Phosphorus fertilizers are regularly applied to get maximum yields. But as a result of the chemical reactions in the soil, these get fixed in the soil resulting only part of it being readily available over the crop period, necessitating, fresh additions. Continuous application of these fertilizers will result in increased concentration of total phosphorus in the soil over time, resulting in large reserves of fixed P. This is an important sink that needs to be tapped for phosphorus nutrition. According to Ozanne (1980), less than 10% of soil P enters the plant-animal cycle. Consequently P deficiency is a widespread problem and P fertilizers are almost universally required for crop production.

Judicious soil management for sustainable agriculture should favour plant health, growth and economic yield gains. Biological means of recovering nutrients in the available form offer an environment friendly sustainable system to support plant and soil health. Microorganisms play a very silent but vital role in maintaining soil nutrient status and structure. Bioinoculants in a major way are represented by these organisms and are used as 'green alternative' to chemical fertilizers. Rhizobacteria cause the release of nutrients into soil solution in naturally balanced proportion (Blake 1993) and exerts beneficial effects on plant development (Glick 1995). The use of such bacteria provides for the development of a production system that is largely organic, maintains and sustains the production base and reduces reliance on expensive imported phosphatic fertilizers. Amongst the various bioameliorants available, the mineral phosphate solubilizing bacteria play an important role in recycling phosphorus in the soil.

Mineral Phosphate Solubilizing Microorganisms

The fixed phosphorus in the soil can be solubilized by certain microbes which have the capacity to convert inorganic unavailable P forms *viz.*, varisite [$Al(OH)_2H_2PO_4$], strengite [$Fe(OH)_2H_2PO_4$], flourapatite [$Ca_{10}PO_4F_2$], hydroxyapatite [$Ca_5(PO_4)_3OH$] and tricalcium phosphate $Ca_3(PO_4)_2$, to forms available for plant growth (Cosgrove 1977). The mineral forms of iron and aluminum phosphates are predominant in acidic soils, while calcium phosphates predominate in neutral to alkaline soils. These unavailable forms are converted to primary orthophosphate (H_2PO_4) and secondary orthophosphates (HPO_4^{-2}), which are available for plant growth. Such microbes are said to possess mineral phosphate solubilizing(MPS) ability (Goldstein 1986.) The MPS microbes, which include bacteria, fungi and actinomycetes, are ubiquitous and their number and type varies with soil, climate and vegetation. Experiments with the MPSB have proved their usefulness on crop growth and yield. Several reviews are available on the phosphate solubilizing microorganisms (Gaur 1990, Rokade & Patil 1992). These earlier reviews covered mainly MPS fungi and the gram-positive bacteria belonging to the genus *Bacillus*. Hence in the present review, we are concentrating on MPS Gram-negative bacteria.

Representative genera of Gram-negative bacteria reported to have been isolated from different ecological zones have been listed in table 1. The list is by no means exhaustive. The ability of these bacteria to solubilize mineral phosphates is generally screened on a solid medium containing insoluble phosphate source such as tricalcium phosphate (TCP), hydroxyapatite or dicalcium phosphate. Traditionally, the MPSB have been isolated on media in which insoluble phosphates like dicalcium phosphate or tricalcium phosphate is used as a sole source of phosphorus. Indicator medium containing bromothymolblue (BTB) also has been developed to enhance chances of picking up efficient bacteria (Krishnaraj 1996). Most of these strains perform well in pot culture condition but their performance in the field may not be consistent. Among the Gram-negative bacteria, *Pseudomonas* has been encountered as the most common solubilizer of mineral phosphates. A clear zone around the colony indicates the ability of release of Pi from the precipitate of insoluble phosphate. More vigorously, the cultures are tested for their Pi release in liquid medium containing insoluble phosphates. The release of Pi is tested spectrophotometrically (Gaur 1990).

Table 1 *Representative mineral phosphate solubilizing Gram-negative bacteria isolated from different ecologica niches*

Ecological niche	Bacteria	Reference
Soil	*Pseudomonas* sp *Pseudomonas striata*	Vidhyasekaran 1973 Arora & Gaur 1979
Coconut plantation soil	*Pseudomonas* sp *Corynebacterium* sp	Thomas & Shantaram 1986
River sediments	*Enterobacter* sp *Pseudomonas* sp	Al Ghazali et al. 1986
Maize root	*Pseudomonas liquifaciens* *Pseudomonas* sp	Sargeeva 1962
Rhizosphere	*Xanthomonas* sp *Flavobacterium* sp	Swaby & Sperber 1958
Groundnut root nodules	*Rhizobium* sp.	Vidhyasekharan 1973
Bamboo rhizosphere	*Serratia* sp	Maheshkumar 1997
Bamboo endorhizosphere	*Enterobacter cloaecae*	Maheshkumar 1997
Bamboo rhizosphere	*Burkholderia cepacia*	Maheshkumar 1997
Sugarcane endorhizosphere	*Acetobacter* sp	Santhi 1998
Lucerne root nodules	*R. meliloti*	Abd-Alla 1994
Lupin root nodules	*R. lupini*	Gostkowska 1976
Pea root nodules	*R. leguminosaram*	Gostkowska 1976
Mung root nodules	*Rhizobium* sp	Shingte et al. 1987
Soybean root nodules	*R. japonicum*	Reichlova 1972
Forest soil	*Pseudomonas* sp	Illmer & Schenner 1992
Rhizosphere/compost	*Enterobacter aerogenes*	Thakkar et al. 1993
Wheat rhizosphere	*Enterobacter agglomerans*	Kim et al. 1997
Soil	*Azotobacter chroococcum*	Kundu & Gaur 1980

Table 2 *Percentage P solubilized by different Gram negative MPS bacteria*

Organism	Source of P	% P solubilized	Reference
Rhizosphere isolates	TCP	4.36– 9.65	Sen & Paul 1957
Rhizosphere isolates	TCP	20.4 – 59.4	Sunder Rao & Sinha 1963
Pseudomonas striata	Mussorie rock phosphate	5.26	Arora & Gaur 1979
Rhizosphere isolates	TCP	19.5– 54	Thomas & Shantaram 1986
Pseudomonas striata	TCP	24 – 58.4	Gaur 1990

TCP, Tricalcium phosphate

Such analysis of the different MPSB showed that the P solubilized varied from 4.36 to 59.4% (table 2). Strains of *Pseudomonas* spp. are capable of releasing 160.5 to 162.5 µg/ml Pi in the medium containing tricalcium phosphate (Santhi 1998). Strains of *Enterobacter agglomerans,* can release Pi ranging from 82.6 to 551.3 µg/ml in medium containing hydroxyapatite (Kim et al. 1997).

It has been recently observed that strains of *Acetobacter* released 142 to 431 µg/ml Pi from tricalcium phosphate (Maheshkumar et al. 1999). This finding extends the possibility of selective use of *Acetobacter* to bioameliorate P in sugarcane rhizosphere. Thus, tremendous diversity exists in the ability to solubilize insoluble phosphates amongst the isolates. The results also indicate the importance of continuous screening and selection for obtaining more efficient isolates. It has been noted that the soil buffering capacity could reduce the acidification of the rhizosphere soil, which could in turn reduce the efficiency of MPS bacteria. Therefore, it has been suggested to buffer the isolation medium with Tris (Subbaram et al. 1998, Gyaneshwar et al. 1998b). It was also noticed that low concentrations of Pi repress the MPS activity (Krishnaraj 1996). Hence, one needs to look for bacteria capable of solubilizing the insoluble phosphates in the presence of external Pi also. This would enable the MPS bacteria to release more Pi into the rhizosphere for plant uptake.

Agronomic Significance of MPSB

Several studies have shown plant growth responses and increased Pi uptake on addition of rock phosphate along with phosphate solubilizing microorganisms to soils. (Rachewad et al. 1992). The agronomic influence of some commonly used species of Gram-negative bacteria has been listed in table 3. A detailed review on the agronomic significance of MPSB has been given by Gaur (1990). Incorporation of these bacteria along with rockphosphate resulted in increased availability of Pi for plant utilization (Hebbara & Suseeladevi 1990, Rachewad et al. 1992, Jisha & Alagawadi 1996). It was observed that inoculation of MPSB along with application of 17.5 kg P/ha as Mussorie rock phosphate resulted in increased dry matter in chickpea and was as effective as single super phosphate (Prabhakar & Saraf 1990). However, field experiments have been rather inconsistent (Kucey et al. 1989). Since bioinoculants are biological entities, variations are expected due to the environmental influences. However, serious thoughts need to be given to this area and methods of improving the efficiency of the applied bioinoculants needs to be looked into. Alternatively, one can look for methods to enrich the soil, in order to raise the natural level of these MPSB. When the current emphasis is on organic farming, the use of the MPSB gains more importance. There is also a need for concerted efforts to explore the bacterial diversity and isolate efficient and competitive MPS bacteria. The stability of these bacteria with respect to their retention of original activity has been found to be weak. Prolonged storage and periodical subculturing has resulted in decreased efficiency of solubilization even in pure culture conditions. Hence, ways of maintaining the stability of their activity is another area that needs attention.

Mechanism Of Mineral Phosphate Solubilization (MPS) By Gram-negative Bacteria

The mechanism of MPS has been a subject of analysis for a long time and is still a matter of curiosity. Organic acids have been implicated to chelate the cationic partners of P ion (Sperber 1958, Katznelson & Bose 1959). Analyses of supernatants of growth of many MPS bacteria have shown the production of mono-, di- and tri-carboxylic acids (table 4). The amount of acids liberated by these bacteria roughly is more than 5% of the carbohydrate consumed (Banik & Dey 1983). A direct correlation between drops in *p*H and increase in available P of the culture media has been observed in certain cases (Sperber 1958, Agnihotri 1970, Liu et al. 1992). In few others, the degree of solubilization was not always proportional to the decline in *p*H (Mehta & Bhide 1970, Wani et al. 1979, Krishnaraj 1987, Asea et al. 1988).

The workers who believe in organic acids theory hardly observed any correlation between the amount of P solubilized and organic acid concentration in the culture medium. Hence it is

Table 3 *Inoculation effect of some Gram-negative mineral phosphate solubilizing bacteria*

Bacteria	Crop	Conditions	Response	Reference
Pseudomonas sp.	Maize	Field	Increased yield	Kavimandan & Gaur 1971
Pseudomonas sp.	Lavender	Greenhouse	Increasded P uptake, plant biomass when inoculated along with rock phosphate	Azcon et al. 1976
Pseudomonas striata	Cotton	Greenhouse	Increased yield	Kundu & Gaur 1980
Azotobacter chroococcum	Cotton	Greenhouse	Increased yield	Kundu & Gaur 1980
Pseudomonas striata	Wheat	Greenhouse	Increased P uptake and dry weight of plant	Gaur 1990
Pseudomonas putida	Canola	Greenhouse	Increased P uptake and yield	Lifschitz et al. 1987
Pseudomonas striata	Chickpea Sorghum	Greenhouse	Increased P uptake and yield	Alagawadi & Gaur 1988
Pseudomonas striata	Rice	Greenhouse	Increased P uptake and yield	Monod et al. 1989
Pseudomonas striata	Sorghum	Greenhouse	Increased P uptake and yield	Jisha & Alagawadi 1996
Pseudomonas sp.	Chickpea	Greenhouse	Increased P uptake and dry matter	Krishnaraj 1996
Enterobacter cloaccae	Bamboo	Greenhouse	Increased dry matter	Maheshkumar 1997
Burkholderia cepacia	Bamboo	Greenhouse	Increased dry matter	Maheshkumar 1997
Serratia marcescans	Bamboo	Greenhouse	Increased dry matter	Maheshkumar 1997

Table 4 *Organic acids secreted by some Gram-negative bacteria*

Bacteria	Organic acids	Reference
Acetobacter sp.	Gluconic	Galar & Boiardi 1995
Escherichia freundii	Lactic	Sperber 1958
Pseudomonas sp.	Citric, Gluconic	Taha et al. 1969
Pseudomonas sp.	Gluconic	Illmar & Schinner 1992
Pseudomonas aeruginosa	Gluconic	Van Schie et al. 1985
Pseudomonas striata	Tartaric, Citric	Gaur 1990
Rhizobium leguminosarum	2-keto gluconic acid	Halder et al. 1991

doubtful as to whether the organic acids are directly and exclusively involved in solubilization (Asea et al. 1988, Parks et al. 1990). Solubilization of calcium phosphate has been reported to occur even in the absence of organic acid (Illmer & Schinner 1992). An HPLC analysis of the culture solution of *Pseudomonas*, in contrast to the expectation did not detect any organic acid even though the bacterium solubilized unavailable forms of P (Illmer & Schinner 1995). In each of these cases, acidification of the medium resulted and it was postulated that H^+ excretion originating from NH_4 assimilation (Parks et al. 1990) and respiratory H_2CO_3 production (Juriank et al. 1986) as an alternate mechanism of mineral phosphate solubilization. These workers also observed a rapid reduction in *p*H and increased availability of Pi in the medium during the initial three days. After the period of glucose utilization, there was maintenance of *p*H (5.5 – 5.8) and a Pi release of 90-105 µg P/ml medium. Utilization of glucose seems to directly correlate with the drop in *p*H. The MPS activity was shown to be due to glucose derived gluconic acid produced in the periplasmic space (Goldstein & Liu 1987, Liu et al. 1992). It was calculated that 60 mM gluconic acid resulted in the release of approximately 0.1 mM Pi. It was hypothesized that the gluconic acid produced lead to the release of protons that finally solubilize the insoluble phosphates (Goldstein 1994). It has been shown that the MPS phenotype is the result of gluconic and 2-keto gluconic acid productions via the direct oxidation pathway involving enzymes located on the outer face of the cytoplasmic membrane. The enzymes include glucose dehydrogenases that oxidize glucose to gluconic acid (Goldstein 1994). The gluconic acid so produced is further oxidized to 2-keto gluconic acid, a very strong naturally occurring organic acid (pKa~2.6) (Goldstein 1995). Thus bio-acidification of the microniche in the vicinity of the root system could play an important role in the release of the locked up phosphates into the soil for plant uptake.

Since the mechanism of P solubilization remained unclear, studies were conducted using MPS^- mutants for the first time (Krishnaraj 1996). The derived MPS^- mutants were compared with their wild type derivatives with respect to the Pi release in the tricalcium phosphate broth, *p*H drop during the same period and identification of organic acid released in the medium. It was found that a highly coordinated reaction occurs leading to dissolution of insoluble phosphate. In the event of phosphate stress, glucose is utilized and gets converted to organic acids that provide H^+ and get co-transported into the external milieu with $H_2PO_4^-$ or HPO_4^{-2}. These reactions are hypothesized to involve the membrane enzymes and organic acid transporters. Such a parallel has been observed in mitochondria, known to be evolutionarily, a bacterial endosymbiont (Margulis 1971) where in phosphate and decarboxylate transporters which function under a proton gradient exist and they transport Pi with concomitant exchange of monocarboxylic and dicarboxylic acids (Vivekanand et al. 1988). The mechanism of the MPS activity is therefore due to release of organic acids, which could also contribute protons to extensively acidify the medium, ultimately causing the dissolution of mineral phosphates (Krishnaraj 1996). However, refined molecular studies are required to identify the existence of such phosphate transporters and the involvement of electro chemical proton gradient in the MPS activity.

Genetics And Molecular Biology Of MPS Activity

To scavenge Pi from organic phosphate, bacteria have developed an elaborate system that mineralizes the organic P into Pi via enzymes - alkaline and acid phosphates. The regulation of this phenomenon has been extensively studied. Several genes are induced under phosphate starvation and constitute the *Pho* regulation. A number of genes are involved including *PhoA*, the gene that codes for alkaline phosphatase. The *Pho* regulon is activated by positive activator, *Pho* B (Torriaini & Ludtke 1985). The *Pho* B binds to the *Pho* box, which is the sequence shared by regulatory region of *Pho* A, *Pho* B, *Pho* T and *Pst* S and activates from the *Pst* B promoter (Makino et al 1989). *Pho* R protein regulates the *Pho* regulon negatively with excess phosphate and positively with limited phosphate. *Pho* M is postulated to inhibit the *Pho* R product, into an inactive form, *Pho* R^m. *Pho* U exhibits a negative

control in the presence of Pi. The *pst-pho* U region appears to be an operon with a transcription attenuator between *Pho* S and *Pho* T (Wanner 1987). Extensive studies have been done on this system and this review does not permit going into elaborate details. But what is interesting is that, the externally added Pi represses this system indicating physiological regulation. Similarly repression of MPS trait was noticed in the presence of increasing levels of Pi in the medium. 20mM of the Pi completely inhibited MPS activity by *Erwinia herbicola* (Goldstein 1986). Similarly, it was found that externally added K_2HPO_4 inhibited the MPS activity of *Pseudomonas* Psd 201 (Krishnaraj 1996) and also by diverse isolates (figure 2) of Gram-negative bacteria (Santhi 1998).

The phosphate stress induced switching on the MPS activity and the repression of MPS activity by externally added Pi is a classic example of physiologically regulated gene expression in bacteria capable of MPS activity. Such observations made it possible to propose the existence of *mps* genes in *E. herbicola* (Goldstein 1986). The model of the phosphate starvation inducible *mps* genes is given in figure 3. Shot gun cloning experiments using *Erwinia herbicola* DNA enabled Goldstein and Liu (1987) to clone gene(s) to convert MPS^- *E. coli* to MPS^+ phenotype. On screening a cosmid pHC76 library from *E. herbicola* they found that a 55 kb insert was able to transform *E. coli* HB 101 to MPS^+ phenotype. Transposon mutagenesis directed to the region of pMCG 898, a cosmid construct carrying a 4.5 kb insert showed that the essential gene was a 1.8 kb region. On the basis of sequence comparison and minicell analysis, Liu et al. 1992 deciphered that the gene codes for

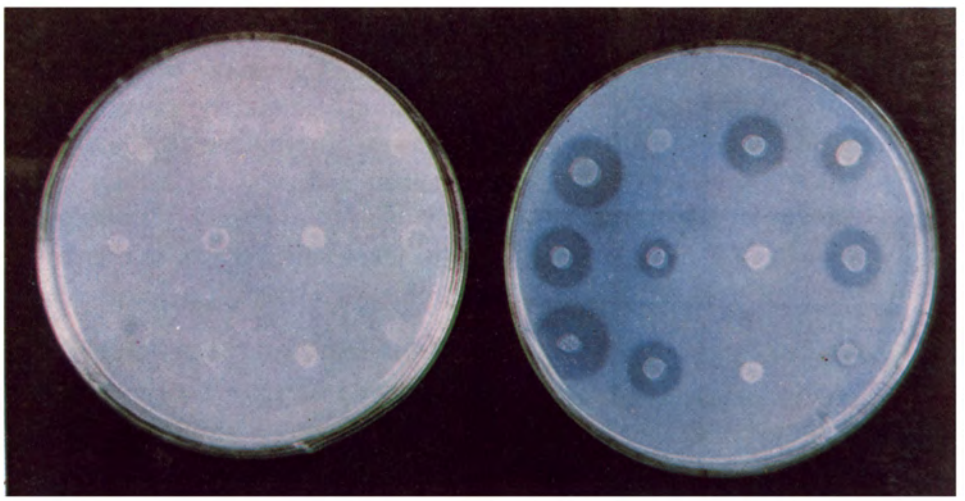

Figure 2 Total repression of mineral phosphate solubilization by K_2HPO_4 (75mM) Note the total absence of any zone (right side plate) of solubilization on modified Sperber's medium containing dicalcium phosphate as insoluble phosphate source) in which K_2HPO_4 was externally added. The left side plate does not have K_2HPO_4.

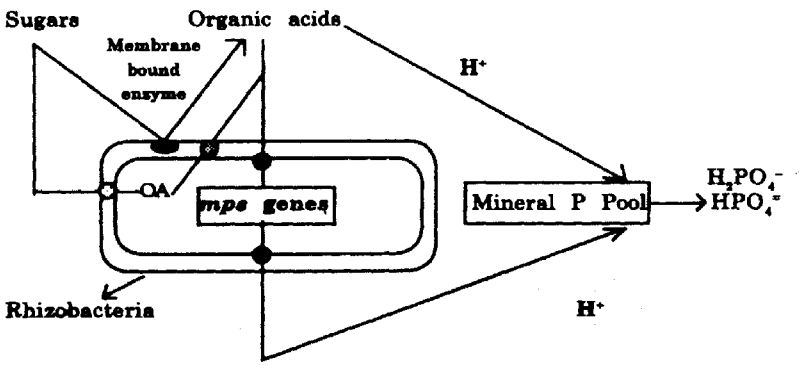

Figure 3 A model of the phosphate starvation inducible mps genes

an enzyme Pyrroloquinoline Quinone (PQQ), a cofactor for the enzyme glucose dehydrogenase (GDH). The quinoprotein GDH controls a unique step in direct oxidation of glucose (Duine et al. 1979). It transfers electron from aldose sugars to the electron transport chain mediated by cofactor PQQ (Duine 1991). Protons generated by the periplasmic oxidation mediated by GDH contribute directly to the transmembrane proton motive force (Van Shie et al. 1985, Duine 1991). All bacteria do not have the full functional complement of GDH enzyme for direct oxidation of glucose. It has been shown that *E. coli*, and *Acinetobacter lowoffi* do not show the production of gluconic acid in the presence of glucose without externally adding PQQ (Goosen et al. 1989). Goldstein and Liu 1987, thus proved that GDH mediated dissimilatory bypass system, involving direct oxidation of glucose to gluconic acid in the periplasmic space was responsible for the mineral phosphate solubilization in *Erwinia herbicola*. Further, they continued their work on transcomplementing MPS$^-$ wild type *E. coli* HB101 using genomic library of *Pseudomonas cepacia* (Babu-Khan et al. 1995). Evidence was presented to show that expression of a single 396 base *P. cepacia* open reading frame, *gab* Y, in *E.coli* was enough, to induce MPS phenotype and production of gluconic acid in *E.coli* JM109. The aminoacid sequence of *gab* Y had no apparent homology with the previously cloned direct oxidation pathway genes indicating that the expression of *gab* Y, in some way result in the production of an alternative redox cofactor that can be used by apo-GDH. Kim et al. 1997, too isolated a clone JM 109 (pKKy) through transcomplementing *E.coli* MPS$^-$ wild type JM109 using cosmid (pHC 79) genomic library of *Enterobacter agglomerans*. However, further analysis of this clone has not been reported yet.

The studies so far carried out transcomplemented MPS$^-$ *E. coli* strains, which probably would lead most often to isolation of genes responsible for a functional GDH enzyme. They may not isolate novel structural/regulatory genes responsible for MPS phenomena in diverse bacteria. Hence, it was thought fit to approach the genetic characterization of MPS activity through the forward genetic approach (Krishnaraj 1996), an approach hither to not used in studying the MPS phenomena

Pseudomonas sp. was subjected to random mutagenesis and different mutants were isolated. MPS$^-$ mutants were selected based on the inability of *Pseudomonas* to form solublization zone on modified Sperber's medium (Krishnaraj et al. 1995b). Random Tn5 insertional mutants of the strain were also isolated. These mutants showed different phenotypic classes with respect to metabolic and cell surface properties (Krishnaraj et.al. 1999). Gene bank of the MPS$^+$ wild type *Pseudomonas* sp Psd 201 was mobilized from *E. coli* into MPS$^-$ derivative strain, *Psedomonas* Psd 207. Two clones were isolated which could restore MPS$^+$ phenotype to Psd207 and had an insert of the size 11.8 kb that might contain one or more *mps* loci (Krishnaraj et al. 1998).

A different approach was also used to analyze and express *mps* gene in MPS$^-$ *E. coli* HB 101 using R plasmid. These R plasmids have been referred to as having chromosome mobilization ability (cma$^+$). Plasmid, R68.45, a derivative of R68 of the Inc P-1 group is an R plasmid isolated from *Pseudomonas aeruginosa* (Haas and Holloway, 1976). It mobilizes bacterial chromosome from a number of different origins and has been used to construct R prime library of the genome for genetic analysis in *Klebsiella, Pseudomonas* and *Rhizobium* (Holloway, 1979). Using this plasmid, R prime library of *Pseudomonas* Psd 201 was constructed and the clones were studied for expression of MPS activity. Two R primes R′ 42 and R′ 45 expressed *mps* gene(s) in *E. coli* DH5 α showing solubilization zone on tricalcium phosphate medium. These R primes are now a source of *mps* gene(s) which could be isolated and characterized (Krishnaraj et al. 1995a). Recently *mps* gene has also been isolated thorough transformation of *E. coli* genomic library of *Synechocytis* (Gyaneshwar et al. 1998a). However much needs to be done on the genetic basis of MPS activity.

The nature of repression of MPS activity by external supplementation of Pi, similar to the repression of *Pho* regulon by K_2HPO_4 indicates the existence of regulatory controls common to the *Pho* regulon and *mps* genes. Molecular studies through isolation of *mps* gene via forward genetics approach and sequence analysis will go a long way in identification of the common controls.

Conclusion

Phosphorus is an essential mineral macronutrient for plant growth. The mineral, however to a large extent, is available in the soil in the insoluble fixed form. The conversion of such forms into orthophosphate anion is done in the soil by certain microbes and the phenomenon is referred to as mineral phosphate solubilization (MPS). Such biophores capable of converting insoluble form of mineral phosphates into primary and secondary orthophosphates are referred to as mineral phosphate solubilizing bacteria (MPSB). These bacteria to a large extent are Gram-negative and have been largely used in bioameliorating Pi. The agronomic importance of such MPSB is well known; although inconsistencies in field experiments have been reported probably due to competitive inability of the introduced strains. Hence, competitive strains capable of stable MPS activity in the rhizosphere are required. Such MPS strains can be mixed with rock phosphate and inoculated into the soil.

To develop better strains of MPSB, two approaches could to be followed *viz.*, (i) to screen extensively the rhizosphere bacteria and select very efficient and competitive strains (ii) to genetically manipulate the available MPS strains to enhance the basal MPS activity. The first approach is a continuous one and is being done in several laboratories. However, genetic enhancement of the basal MPS activity requires in depth genetic analysis of the phenomenon. Genes responsible for MPS phenomenon have been isolated through trans-complementation of MPS$^-$ *E. coli*. However, the forward genetics approach of isolating MPS$^-$ mutants of wild type and trans-complementation analyses using genome library of the wild type is lacking and needs to be emphasized to identify the structural as well as regulatory genes involved in MPS activity. Common regulatory controls between the *Pho* regulon, the well-studied phosphate starvation inducible system and *mps* genes need to be identified. The genes thus identified could be used to develop strains to bioprocess phosphate from rockphosphate using continuous flow stirred tank bioreactor as shown in a very elegant earlier report (Goldstein et al. 1993). Such genes could be also used to develop agronomically more efficient MPS bacteria and transgenic bacteria capable of multiple agronomic benefits. The ultimate goal is to develop highly efficient and competitive MPS strains to inoculate the soil along with the rock phosphate ores in order to see that plants receive timed release of phosphate throughout the growth period of the plant.

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