Proc Indian Natn Sci Acad **80** No. 2 June 2014 Spl. Sec. pp. 389-405
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DOI: 10.16943/ptinsa/2014/v80i2/55116

Review Article

Mineral Phosphate Solubilization: Concepts and Prospects in Sustainable Agriculture

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(Received on 08 March 2013; Revised on 23 August 2013; Accepted on 14 September 2013)

Microorganisms play very important role in biogeochemical cycle in soil, which reflects the plant growth and influence the agricultural production. Phosphate solubilizing microorganisms (PSM) are very important to the plants under phosphorus stress. As we know, Phosphorus is one of the essential macronutrients, along with nitrogen, required by the plants for their vital functions and survival. But, the efficiency of phosphatic fertilizers is very low due to their fixation in both acidic and alkaline soils which are predominant in India. Therefore, the inoculation of mineral phosphate solubilizers and other useful microbial inoculants in these soils would play an important role to restore the overall balance of nutrients and health of the soil to sustain it for posterity. The molecular genetics and mechanism of mineral phosphate solubilization and their efficiency in releasing phosphates for plant uptake is seemingly different and varies with microorganisms. Hence, the isolation and characterization of superior strains of mineral phosphate solubilizers to suit different soil types is imperative. These phosphate-solubilizing bacteria have the capacity to convert the insoluble forms of phosphorus into its soluble form, which ultimately gets available to plants, a phenomenon often referred to as mineral phosphate solubilisation. The combination of chemical fertilizers with this beneficial microorganism is also one of the ways to increase the agricultural yield without loss of nutrients. Moreover, the developments of commercial bioinoculants will be greatly accepted by farmers and will help to maintain agriculture sustainability. The present article describes the progress of research in this area and future insights about use of such biophores in agriculture.

Key Words: Mineral Phosphate Solubilization; Molecular Genetics; Organic Acids; Direct Oxidation Pathway

Introduction

The rapid industrialization, urbanization and increase in human population have caused agricultural land shrinkage by nearly 2.76 million hectares in last two decades, resulting in food crisis in India [1] and all over the world. By 2050, the world's population will reach 9.1 billion, 34 per cent higher than today. Hence, the food production must increase by 70 per cent to feed the growing human population. In 2025, the food grain requirement for India's 1.4 billion people will be about 300 million tonnes. This production level will require about 30 million tonnes of nitrogen (N), phosphorus (P), and potassium (K), including 8.6 million tonnes of P_2O_5. In addition, another 14 to 15 million tonnes of NPK would be needed for vegetable, plantation, sugarcane, cotton, oilseed, potato, and other crops. Thus, about 40 to 45 million tonnes of NPK, containing 11 to 13 million tonnes of P_2O_5, will be required just to maintain a broad average $N:P_2O_5:K_2O$ ratio of 4:2:1 [2].

Traditional farming practices were integrated with cultivation of food grains, vegetables and rearing of milch animals. Even in a small unit, facilitated availability and use of organic matter, when

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incorporated into soil, would enable sustainable use of soil for achieving higher yields. The shift to chemical agriculture occurred due to the acceptance of the high yielding hybrids, which were largely responsive to chemical fertilizers but susceptible to pests and diseases. The application of chemical fertilizers in large measures has reduced the organic matter addition to soil. As a result, the consumption of these fertilizers has increased over the years without considerations to their harmfulness to our environment [3]. Poorly managed consumption of resources and poor applications of Pi fertilizers and pesticide products have endangered our environment. Therefore, it is important to use bioinoculants, which are bio-friendly, economically feasible and replaceable sources of Pi, before the world consumption rate will reach to the point of no return in coming future [4].

In order to ensure a reduction in the use of chemical P fertilizers, the use of microorganisms has been advocated and they have been found to play a very important role in the enhancement and sustenance of crop yields and productivity with improvement in soil health. Majority of agricultural soils contain large reserves of phosphorus, of which a considerable part accumulates as consequence of regular and repeated applications of phosphorus fertilizers. The phenomenon of fixation and precipitation of P in soil, which is largely dependent on the soil pH, results in a low efficiency of soluble P fertilizers. In acidic and calcareous soils, P is precipitated as Al, Fe phosphates and Ca phosphates respectively. Microbial activity plays a vital role in increasing the availability of minerals essential for plant growth and ultimately lowers the requirement of synthetic fertilizers [5]. As one of the main challenges in the world of agriculture is the availability of phosphorus (P) for plant nutrition, it requires a closer introspection.

Importance of Phosphorus in Agriculture

Dependence of agriculture on phosphorus and the critical situation the future generations will face if the scarce amount of the un-renewable resource were to run out. Phosphorus (P) is the 11th most abundant naturally occurring element in the earth's crust, water, and all living organisms and is one of the 16 elements that are essential for plant growth in modern agriculture. The role of P in crop production systems is exemplified by the amount of fertilizer-P used during the last 35 years. The amount has doubled since 1960, stabilizing at slightly under two million tons/year over the last 10 years.

Functions of Phosphorus in Plants

Phosphorus is a component of many cell constituents and it plays a major role in several key processes, including photosynthesis, respiration, storage and transfer of energy, cell division, and cell enlargement. Adequate phosphorus is needed for the promotion of early root formation and growth. Phosphorus also improves crop quality and is necessary for seed formation [6]. It is required for seed germination, photosynthesis, and is essential for flower and fruit formation. In photosynthesis and respiration, P plays a major role in energy storage and transfer as ADP and ATP (Adenosine di- and triphosphate) and DPN and TPN (di- and triphosphopyridine nucleotide). P is part of the RNA and DNA structures, which are the major informational biopolymers. P is required in large quantities in young cells, such as shoots and root tips, where metabolism is high and cell division is rapid. P aids in root development, flower initiation, and seed and fruit development. P has been shown to reduce disease incidence in some plants and has been found to improve the quality of certain crops. Because P is needed in large quantities during the early stages of cell division, the initial overall symptom is slow, weak, and stunted growth. In a mature plant, seeds have the highest concentration of P [7].

P is relatively mobile in plants and can be transferred to sites of new growth, causing symptoms of dark to blue-green colouration to appear on older leaves of some plants. Under severe deficiency, purpling of leaves and stems may appear. Lack of P can cause delayed maturity and poor seed and fruit development [8].

Status of Phosphorus in Agricultural Soils

Different cultivation intensive agricultural practices and irrigation have significantly disturbed soil nutrition balance. Nitrogen and phosphorus that may be available to plants are present in millimolar and micromolar amounts in soil [9]. Phosphorus is present at levels of 400-1200 mg/kg of soil [10]. The quantity of P in the soil solution, even when at relatively high levels, is only in the range of 0.3 to 3.0 kg/ha (0.3-3.0 lb/ac) [11], a large fraction of this is in an insoluble form and only <10 % enters the plant-animal cycle [12]. It has been estimated that at any point of time, the dissolved/available forms of P in many soils may only be from 0.01-0.06 ppm (0.02-0.12 lb P/acre). Organic and inorganic phosphates are the two forms of phosphorus present in soil.

The first systematic soil fertility map of Indian soils was given in 1967 by Ramamurthy and Bajaj [14], which indicated that 4% samples were high in available P. The soil fertility map published in 2002 [15], however, indicates that around 20% of soil samples are high in available P, indicating accumulation of phosphorus in soil. The recently prepared GIS based district-wise soil fertility maps of India [16] showed that soils of about 51% districts were low, 40% were medium and 9% were high in available P. The high P status in some soils may be due to non-judicious use of phosphatic fertilizers by the farmers and its subsequent fixation and accumulation in soil [17].

P Requirement of Plants

The P requirement of plants varies considerably. Tree crops have relatively low P requirements with the critical values ranging from 0.12 to 0.15 per cent. Grasses have higher P requirements with critical values ranging from 0.20 to 0.25 per cent. Legumes and some vegetable crops have relatively higher P requirements with critical values being 0.25 to 0.30 per cent or slightly higher [18].

Phosphorus deficiencies normally occur early in the growth cycle of the plant, when the P requirement is high. The P content of plants is initially high and declines with age and since P is a fairly mobile element in plants, deficiencies generally occur on older tissue. The excess range of P is not clearly known. P level in young plants can be very high, such as 0.50 to 1.00 per cent, but these high levels may reflect actual need. In some instances, high P plant levels may cause imbalances and deficiencies of other elements, such as Zn, Cu, Fe, etc. Plant P thus needs to be maintained within the sufficiency range by proper P fertilization and microbially mediated transformation of nutrients in soil.

P Supply and Demand

It is often mentioned that there is an "impending shortage" of phosphorus fertilizers, although phosphorus constitutes 0.1% of the lithosphere and the supplies are likely to outlast our species and possibly even the planet itself. Only about 0.5 parts per million of phosphorus occurs in phosphate rock deposits that can be extracted economically and the richest deposits are rapidly being depleted. The price of phosphate rock has risen four-fold in 10 years. Undoubtedly, new reserves are likely to be found in the future, but most recent discoveries have occurred in just a few places, mostly Morocco and the Western Sahara with the former alone contributing to more than 70% of documented phosphate on earth [19].

The demand for phosphorus increased sharply in the mid-20th century with the success of the green revolution, when plant breeders successfully produced higher-yield versions of familiar field crops. Those higher yields required larger doses of fertilizers. An estimated 17 million tons of processed phosphorus will be used on the world's farm fields this year, and the demand is only expected to rise in the forthcoming years. The increase in world's population, an expanding global affluence and the resulting increased demand for more food, particularly meat, will only put more stress on the accessible supplies of phosphorus. Dana and her colleagues in 2009 [20] estimated that the amount of phosphorus that people consume in food is only one-fifth that being mined, suggesting that huge amounts simply escape.

Role of Microbes in Biogeochemical Cycle of P in Soil

Microorganisms play a fundamental role in the biogeochemical cycling of inorganic and organic P in the rhizosphere [21-27], with a significant percentage of the total culturable bacterial and fungal communities being reported to have inorganic P solubilizing activity [28-31].

Rhizosphere microbial inoculants have been proposed as components of integrated nutrient management systems [24, 26, 32] with specific interest in their ability to increase the availability of P for crops [23, 28, 29]. Research on crop inoculants has focused largely on introducing free-living microorganisms that form non-specific, beneficial associations with a range of plant hosts that can be mass produced, and have potential to persist in the rhizosphere [24, 26, 29, 33]

Microorganisms involved in the solubilization of insoluble phosphorus include bacteria, fungi, and actinomycetes [34-37]. Of total microbial load, 1-50% of bacteria and 0.1-0.5% of fungi have been hypothesized to be capable of solubilizing insoluble inorganic phosphate [30]. Both, Gram negative as well as Gram positive bacteria are capable of mineral phosphate solubilization. But, Gram negative bacteria have been characterized nowadays for this mechanism.

Although symbiotic associations with AM fungi are recognized as playing an important role in the P nutrition of many plants, particularly in low P soils [23, 38], the inability to readily culture AM fungi in artificial media and lack of establishment of any host plant specific associations has limited their development as rhizosphere inoculants. Most research into the development of microbial inoculants to enhance P availability and root uptake has centred on soil microorganisms capable of solubilizing sparingly-available P [22, 28, 39].

However, when PSB was used in combination with P fertilizers, a much greater effect on the Pi uptake and growth was observed. Genera such as *Alcaligenes, Acinetobacter, Arthrobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Paenibacillus, Pseudomonas, Rhizobium,* and *Serratia* have been identified, analyzed and tested.

Pseudomonas, *Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers in the soil [42]. Recently, *Cupriavidus basilensis*, which was reported to have the capability of copper chelation, was found for the first time to have the ability to mineralize inorganic as well as organic phosphorus [88].

A thorough understanding of the rhizosphere niche, microbial community interactions and genetic mechanisms associated with enhancing P-availability in ‘responsive’ soils will assist development of inoculants across farming systems, with potentially greater consistency in performance. The efficacy of P-solubilizing inoculants depends on their capacity to colonize, survive and multiply in the rhizosphere [89-90].

Molecular genetics also provides opportunity for elucidation of the mechanisms associated with P solubilization and plant growth promotion [91]. Comparative genomic and transcriptomic sequencing of related microbial genotypes, with and without P-solubilizing capabilities and differential gene expression analyses of strains growing under conditions that require P-solubilization for growth, have potential to identify enzymes, metabolites and transport proteins involved in these processes. With regard to *Penicillium* spp., comparison of sequence databases will assist the genomic annotation of species with mineral phosphate solubilizing and plant growth promoting functions that could eventually lead to enhancement of P availability and use by the crop plants.

Mechanisms of MPS Activity

Several soil microorganisms from diverse ecological niches have the ability to solubilize insoluble mineral phosphate into the ionic forms that can be taken up by the crop plants. Some of the different mechanisms of phosphate solubilization in different microbes are considered below.

Organic Acid Production

The major mechanism of mineral phosphate solubilization as observed in different microbes is

Table 1: Phosphate solubilizing microorganisms

S.No.	Strains	MPS potential	References
1	*Acetobacter diazotropicus*		[39]
2	*Acetobacter liquefaciens*	72.9 mg/ml	[40]
3	*Acetobacter* sp.	63.8 mg/ml	[40]
4	*Achromobacter xylosoxidans*		[41]
5	*Acinetobacter* sp.	334-443.26 µg/ml	[42, 43]
6	*Aerobacter aerogenes*		[42,44]
7	*Agrobacterium radiobacter*		[45]
8	*Agrobacterium* sp.		[42,44,46]
9	*Alcaligenes* sp.		[42,44]
10	*Arthobacter mysorens*		[45]
11	*Azotobacter chroococcum*	1.10-98.11 µg/ml	[47]
12	*Bacillus brevis*		[48]
13	*Bacillus* sp.	236-395mg/ml	[47,49]
14	*Bradyrhizobium japonicum*		[50]
15	*Brevibacterium* sp.		[18,44]
16	*Burkholderia anthina*	>600 µg/ml	[51-56]
17	*Burkholderia cepacia*	250-375mg/ml	[42,57]
18	*Burkholderia* sp.	0-200 µg/ml	[58,59]
19	*Burkholderia tuberum*		[60]
20	*Cladosporium herbarum*		[61]
21	*Corynebacterium* sp.		[44,62]
23	*Enterobacter agglomerans*		[63]
24	*Enterobacter aerogenes*		[64]
25	*Enterobacter asburiae*		[65]
26	*Enterobacter cloacae*		[66]
27	*Enterobacter intermedium*		[67]
28	*Enterobacter* sp.	568-642µg/ml	[68,69,70]
29	*Erwinia herbicola*		[71,72]
30	*Escherichia freundii*		[44,62]
31	*Flavobacterium* sp.		[73]
32	*Gluconacetobacter* sp	180 µg/ml	[58,69]
33	*Gluconobacter diazotrophicus*		[74,75]
34	*Micrococcus* sp.	122.4-396.57 µg/ml	[42,76]
35	*Mycobacterium* sp.		[73]
36	*Paenibacillus kribensis*		[77]
37	*Pantoea agglomerans*	62.76-338 mg/ml	[51,52,54, 56,80-83]
38	*Pseudomonas aeruginosa GES-18*		[81]
39	*Pseudomonas cepacia*	35 mg/ml	[82]
40	*Pseudomonas chlororaphis*	493 µg/ml	[83]
41	*Pseudomonas fluorescens*	322-520 µg/ml	[62,44, 51,84]
42	*Pseudomonas gladioli*	68.8 mg/ml	[41]
43	*Pseudomonas pinophillum*		[44,48]
44	*Pseudomonas putida*		[49,83]
45	*Pseudomonas striata*	156 mg/ml	[60]
46	*Pseudomonas syringae*		[48]
47	*Rahnella aquatilis*		[85]
48	*Ralstonia* sp.	189 mg/ml	[57]
49	*Rhizobium leguminosarum biovar Phaseoli*	93-326.4 µg/ml	[86,87]
50	*Corynebacterium* sp.		[63]
51	*Rhizobium meliloti*	120-620µg/ml	[63]
52	*Rhizobium* sp.	155-840µg/ml	[63,75,76]
53	*Serratia marcescens*	188-500mg/ml	[77,83]
54	*Serratia phosphaticum*		[44,62]

(*Contd ....*)

through the release of organic acids. The production of organic acids results in acidification of the microbial cell and its surroundings by decreasing the pH. The amount and type of the organic acid produced vary with the microorganism. The amount of soluble phosphate released depends on the strength and type of acid.

The extracellular oxidation of glucose via the quinoprotein glucose dehydrogenase to gluconic acid is the most efficient mineral phosphate solubilization mechanism in Gram negative bacteria [66, 92-94]. Organic acids contribute to the lowering of solution pH as they dissociate in a pH dependent equilibrium, into their respective anion(s) and proton(s) [95]. Microorganisms often export organic acids as anions [96]. Acid production in laboratory condition in the medium provided with calcium phosphate is indicated by the drop in pH of the growth media and the efficiency of Pi release is dependent on the nature of the acids like aliphatic or phenolic rather than total acidity. A decrease in the pH of the culture filtrate from an initial value of 7.0 to final value of 2.0 has been recorded [97]. Phenolic acids and citric acids are found less effective than aliphatic acids in

P-solubilization. Gluconic acid and 2-ketogluconic acid seems to be the most frequent acid observed to be produced during mineral phosphate solubilization. Other organic acids were also found to produce mixtures of lactic, isovaleric, isobutyric, acetic acid, glyoxalic, oxalic, malonic, fumaric, pyruvic, tartaric and succinic acid etc. (Table 2) [42, 70, 98]. The cell membrane associated pumps help acquire the nutrient aided by the ionic gradient. The proton released from the organic acids complex with the cations.

The carbon source is very important for the nature and type of acid production by the phosphate solubilizing bacteria [103, 106].

Other Theories of Mineral Phosphate Solubilization

Inorganic acids such as sulphuric, nitric, and carbonic acid are considered as other mechanisms for phosphate solubilization produced by some strains [113]. But their effectiveness and contribution to P release in soils seems to be less than organic acid production. Humic and fulvic acids released during microbial degradation of plant debris are also good chelators of calcium, iron and aluminium present in insoluble phosphates [114]. In certain cases, phosphate solubilization is induced by phosphate starvation [115]. Solubilization without acid production has been hypothesized due to the release of protons accompanying respiration by ATPase activity or ammonium assimilation [116-118].

More solubilization occurs in presence of ammonium salts than nitrate salts as the nitrogen source in the media. Besides, the production of chelating substances H_2S, CO_2, mineral acids, siderophores, biologically active substances like indole acetic acid [119], gibberellins and cytokinins

Table 2: Organic acid production by different phosphate solubilizing microorganisms

Bacterial and fungal Strains	Organic acid	References
Acetobacter sp.	Gluconic acid	[99]
Aspergillus flavus *Penicillium* sp. *and A.niger*	Gluconic, fumaric, succinic, acetic, oxalic, citric	[100]
Aspergillus niger	Gluconic acid	[101]
Burkholderia cepacia Burkholderia sp.,	Gluconic acid	[102]
Serratia sp., *Ralstonia* sp., *Pantoea* sp.	Gluconic acid	[71]
Citrobacter sp.	Gluconic acid	[103]
Enterobacter sp.	Gluconic, succinic, acetic, glutamic, oxaloacetic, pyruvic, malic, fumaric, alpha-ketoglutaric	[57]
Escherichia freundii	Lactic	[104]
Penicillium bilaii	Citric and oxalic acid	[105]
Penicillium regulosum	Citric and gluconic acid	[106]
Pseudomonas aeruginosa	Gluconic	[97]
Pseudomonas putida	Gluconic acid	[107]
Pseudomonas sp.	Citric, gluconic	[108]
Pseudomonas sp.	Gluconic	[98]
Pseudomonas sp.	2-ketogluconic acid	[109]
Pseudomonas striata	Tartaric & citric	[98]
Rhizobium leguminosarum	2-ketogluconic acid	[110]
Serratia marcesence	Gluconic acid	[111]
Sinorhizobium meliloti	Malic, succinic and fumaric	[112]
Stenotrophomonas maltophilia	Gluconic acid	[59]

[120] have been correlated with phosphate solubilization. Chelation involves the formation of two or more coordinate bonds between an anionic or polar molecule and a cation, resulting in a ring structure complex [22].

Recently, it has been published that the RD64 strain, a *Sinorhizobium meliloti* 1021 strain engineered to overproduce indole-3-aceticacid (IAA), showed improved nitrogen fixation ability compared to the wild-type 1021 strain. It also showed high effectiveness in mobilizing P from insoluble sources, such as phosphate rock [121].

Genetics of Direct Oxidation Pathway and Organic Acid Production by Soil Microbes

The characterization of different phosphate solubilizing bacteria has shown that direct oxidation pathway provides the biochemical basis for highly efficacious phosphate solubilization in Gram negative bacteria via diffusion of the strong organic acids like gluconic acid produced in the periplasm into the adjacent environment [59, 93-95,101,103,107]. Glucose is the precursor for synthesis of gluconic acid [122]. This has suggested that phosphate solubilization in these strains is mediated by glucose or gluconic acid metabolism. Glucose metabolism has a different pathway, which is shown in Fig. 1.

The production of organic acids is considered as the principal mechanism for mineral phosphate solubilization in bacteria that has been correlated with the cloning of two genes involved in gluconic acid production *viz*., *pqq* and *gabY* which direct the dissolution of hydroxyapatite in assay media [82, 123]. Gluconic acid is the principal organic acid produced by *Pseudomonas* spp., *Erwinia herbicola*, *Bacillus* spp., *Burkholderia* spp. and *Rhizobium* spp. Other organic acids such as lactic, isovaleric, isobutyric, acetic, glycolic, oxalic, malonic and succinic acids are also generated by different phosphate solubilizing bacteria. It has been very well

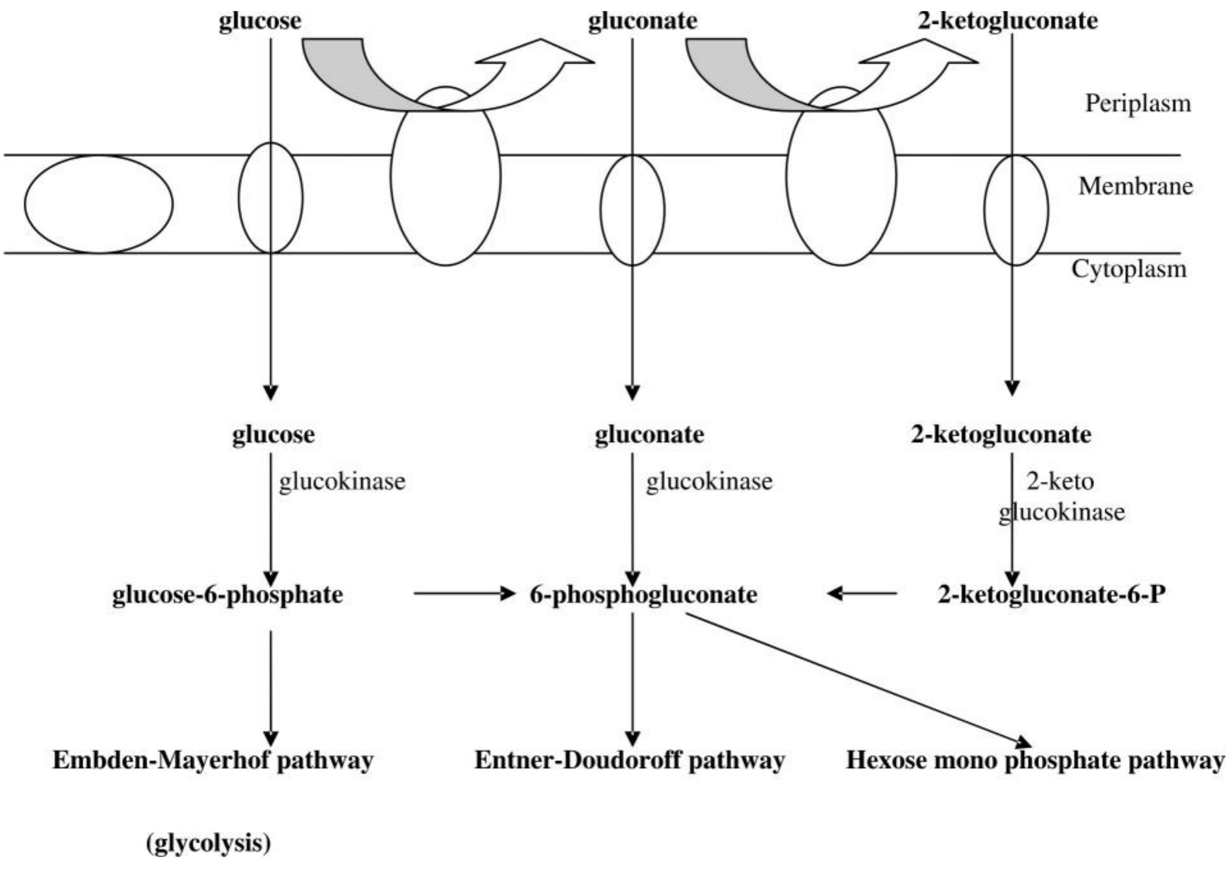

Fig. 1: Alternative pathway in glucose metabolism

shown that an efficient mineral phosphate solubilizing phenotype in Gram-negative bacteria resulted from extracellular oxidation of glucose to gluconic acid via. Quinoprotein glucose dehydrogenase equipped with pyrroloquinoline quinone (PQQ) as a cofactor. A *pqq* gene cluster producing PQQ was detected in *E. intermedium* and this sequence conferred phosphate-solubilizing activity to *E. coli* DH5α. The 6,783 bp PQQ sequence had six open reading frames (pqqA, B, C, D, E and F) and showed 50-95% homology to pqq gene of other bacteria. Gluconic acid seems to be the most frequent agent of mineral phosphate solubilization along with 2-ketogluconic acid as another organic acid identified in strains with phosphate solubilizing ability.

Genes involved in PQQ biosynthesis have been cloned from several organisms. Five *Acinetobacter calcoaceticu*s *pqq* genes, pqqIV, V, I, II, and III [124] and six *Klebsiella pneumoniae pqq* genes, *pqq*A, B, C, D, E, and F [125], were cloned and comparison of the deduced amino acid sequences showed that the proteins encoded by the first five genes of the *K. pneumoniae pqq* operon (*pqq*ABCDE) show similarity to the proteins encoded by the corresponding *A. calcoaceticus* genes (49 to 64% identical amino acid residues). The *K. pneumoniae pqq*F gene encodes a protein that shows similarity to *E. coli* protease III and other proteases, but its equivalent has not yet been found in *A. calcoaceticus*. Three *Methylobacterium extorquens AM1 pqq* genes, *pqq*D, G, and C, have been cloned and sequenced [126]; *pqq*C was only partly sequenced. The encoded proteins showed similarity to the *K. pneumoniae* PqqA, B, and C proteins and the *A. calcoaceticus* PqqIV, V, and I proteins, respectively. Four additional *pqq* genes have been detected in *M. extorquens* by isolation of mutants and complementation studies. From similar studies, six (possibly seven) *pqq* genes have been postulated in *Methylobacterium organophilum DSM760*. A DNA fragment cloned from *Erwinia herbicola* contained a gene encoding a protein similar to *K. pneumoniae* PqqE and *A. calcoaceticus* PqqIII [127]. Except for the *K. pneumoniae* PqqF protein, none of the Pqq proteins shows similarity to other proteins in the database. One of the *pqq* genes is small and may encode a polypeptide of 24 amino acids (PqqIV, *A. calcoaceticus*), 23 amino acids (PqqA, *K. pneumoniae*), or 29 amino acids (PqqD, *M. extorquens AM1*). Interestingly, these putative polypeptides contain conserved glutamate and tyrosine residues (positions 15 and 19, respectively, in *K. pneumoniae* and the equivalents in *A. calcoaceticus* and *M. extorquens*). Those residues have been suggested previously as precursors in PQQ biosynthesis. Replacement of Glu-16 by Asp and Tyr-20 by Phe in *A. calcoaceticus* PqqIV abolished PQQ biosynthesis. A frame shift in *K. pneumoniae pqq*A had the same result. It was suggested that the PqqA/PqqIV polypeptide might act as a precursor in PQQ biosynthesis [124-127].

A mutant, K818, defective in plant growth promotion was identified through mutagenesis in *Pseudomonas fluorescens* B16. This mutant was transcomplemented by a cosmid clone, pOK40, and transposon mutagenesis directed to the clone gene/s showed that the genes responsible for plant growth promotion reside in a 13.3-kb BamHI fragment, which was sequenced and identified to be seven known and four previously unidentified pyrroloquinoline quinone (PQQ) biosynthetic genes [128]. Another gene (*gabY*) involved in MPS was cloned from *Pseudomonas cepacia* [82]. The deduced amino acid sequence showed no homology with previously cloned direct oxidation pathway genes, but was similar to histidine permease membrane-bound components dehydrogenase (gcd) gene. Babu-Khan *et al*. [82] hypothesized that this ORF could be related to the synthesis of PQQ by an alternative pathway, or the synthesis of a GCD co-factor different from PQQ. In addition, a DNA fragment from *Serratia marcescens* induces gluconic acid synthesis in *E. coli*, but shows no homology to PQQ or GCD genes [111]. They suggested that this gene acted by regulating gluconic acid production under cell-signal effects.

Other isolated genes involved in the MPS phenotype seem not to be related with pqq DNA or gcd biosynthetic genes. A genomic DNA fragment from *Enterobacter agglomerans* showed MPS activity in *E. coli* JM109, although the pH of the medium was not altered [85]. These results indicate that acid production is an important way, but not the only mechanism, of phosphate solubilization by bacteria [129]. More recently, a phosphoenol pyruvate carboxylase (*pcc*) gene from *Synechococcus* PCC 7942 appears to be involved in MPS [130]. All these findings demonstrate the complexity of MPS in different bacterial strains. The *Synechococcus elongatus* PCC 6301 phosphoenolpyruvate carboxylase (*ppc*) gene was constitutively overexpressed in fluorescent pseudomonads to increase the supply of oxaloacetate, a crucial anabolic precursor and an intermediate in biosynthesis of organic acids implicated in phosphate (P) solubilization. *Pseudomonas fluorescens* ATCC 13525, transformed with pAB3 plasmid containing the *ppc* gene showed a 14-fold increase in PPC activity under P-sufficiency resulting in increased carbon flow through the direct oxidative pathway and reduced metabolic overflow. Under P-limitation, contribution of the direct oxidative pathway significantly increased in *P. fluorescens* ATCC 13525. However, *ppc* overexpression enhanced glucose catabolism through intracellular phosphorylative pathway. These results correlated with gluconic, pyruvic and acetic acid levels as well as the activities of key glucose catabolic enzymes. Irrespective of the P-status, *ppc* overexpression improved biomass yield without altering growth rate, resulting in improved P-solubilizing abilities of *P. fluorescens* ATCC 13525 as well as of the wheat rhizosphere fluorescent pseudomonads isolates Fp585, P109 and Fp315. Collectively, *ppc* overexpression reversed the P-status dependent glucose distribution between the direct oxidative and phosphorylative pathways of glucose catabolism in *P. fluorescens* ATCC 13525 and presented a feasible genetic engineering approach for developing efficient P-solubilizing bacteria [131].

Expression in *E. coli* of the mps genes from *Ranella aquatilis* supported a much higher GA production and hydroxyapatite dissolution in comparison with the donor strain. MPS mutants of *Pseudomonas* spp. showed pleiotropic effects, with apparent involvement of regulatory mps loci in some of them [39]. This suggests a complex regulation and various metabolic events related to this trait. Expression of an mps gene in a different host could be influenced by the genetic background of the recipient strain, the copy number of plasmids present and metabolic interactions. Thus, genetic transfer of any isolated gene involved in MPS to induce or improve phosphate-dissolving capacity in PGPB strains, is an interesting approach. An attempt to improve MPS in PGPB strains, using this approach, was carried out [42] with a PQQ synthetase gene from *Erwinia herbicola*. This gene, isolated by Goldstein and Liu [71], was subcloned in a broadhost range vector (pKT230). The recombinant plasmid was expressed in *E. coli*, and transferred to PGPB strains of *Burkholderia cepacia* and *Pseudomonas aeruginosa*, using tri-parental conjugation. Several of the exconjugants that were recovered in the selection medium, showed a larger clearing halo in medium with tricalcium phosphate as the sole P source. This indicates the heterologous expression of this gene in the recombinant strains, which gave rise to improved MPS ability of these PGPBs. More recently, a genomic integration of the pcc gene of *Synechococcus* PCC in *P. fluorescens* 7942 allowed phosphate solubilization in the recipient strain [132]. In other work, a bacterial citrate synthase gene was reported to increase exudation of organic acids and P availability to the plant when expressed in tobacco roots. Citrate overproducing plants yielded more leaf and fruit biomass when grown under P-limiting conditions, and required less P-fertilizer to achieve optimal growth.

The transgenic *Azotobacter*, expressing *E. coli gcd*, showed improved biofertilizer potential in terms of mineral phosphate solubilization and plant growth-promoting activity with a small reduction in nitrogen fixation ability. *Azotobacter vinelandii* AvOP harbouring pMMBEGS1 and pMMBEPS1, without supplementation of PQQ, showed pink colouration of the colony, solubilized TCP and also released inorganic phosphate in liquid media more than the wild type, suggesting that the *Azotobacter* was able to synthesize the cofactor PQQ. *Azotobacter vinelandii* AvOP genome sequencing project also revealed the presence of ORFs with homology to the PQQ cofactor biosynthetic genes. The wild-type *Azotobacter* was less efficient in solubilizing mineral

Table 3: Influence of mineral phosphate solubilizers on the plant growth and yield

Plant	Inoculation	Effect	References
Chickpea	*Mesorhizobium* sp., *Pseudomonas aeruginosa*	Enhanced grain and straw yield, uptake of P and N, nodulation, dry weight of root & shoot	[165]
Chickpea	*Glomas intraradices, Pseudomonas putida, P. alcaligenes, P. aeruginosa, A.awamori & Rhizobium* sp.	Increase in growth & yield, nodules per root, resistence to pathogen	[164]
Cicer arietinum	*Pseudomonas fluorescences, Bacillus megaterium*	Increase in radical & plumule length, seedling length	[169]
Cotton	*Bacillus* sp.	Increase in plant height, number of bolls per plant & boll weight & soil available P.	[170]
Cowpea	*Microccus* sp.	100 & 39.2% higher root & shoot lengths, higher root growth, increase dry biomass as well as number of roots	[171]
Cowpea	*Gluconacetobacter* sp. and *Burkholderia* sp.	Improved nodulation, root and shoot biomass, straw and grain yield and phosphorous and nitrogen uptake	[58]
Glycine max	*Fluorescent pseudomonas*	Increased nutrient uptake, tolerance to stress, salinity, metal toxicity & pesticide	[172]
Gram	*Rhizobium and PSB namely Pseudomonas striata and Bacillus polymyxa*	Increase in nodulation, nitrogenase activity, dry matter content	[173]
Gram	*Pseudomonas* sp.	Increase in its growth and grain yield	[174]
Lotus tenuis	*Pseudomonas* sp., *Erwinia* sp., *Pantoea* sp., *Rhizobium* sp.	Enhanced growth	[175]
Macroptilium atropurpureum (Siratro)	*Burkholderia tuberum*	Nodulation & effective nitrogen fixation	[176]
Maize	*Rhizobium leguminosarum biovar phaseoli (*MPS$^+$*)*	Increase colonization & dry matter	[86]
Mothbean	*Rhizobium & Pseudomonas* sp.	Increase in shoot & root length	[177]
Rice	*Pseudomonas* sp., *Serratia* sp., *Azospirillum* sp.	Increase in plant growth & P uptake	[176]
Soybean	*Pseudomonas* sp.	Enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake	[150]
Sunflower	*Bacillus* sp.	Increase in growth, yield & quality of plant, oil yield	[178]
Tomato	*Pantoea agglomerans, Burkholderia anthina*	Increased plant height, root length, shoot & root dry weight, phosphorus uptake & available phosphorus content	[56]

phosphate with a single copy of *gcd* in the genome (GenBank Accession No. NZAAAU02000003) [133]. A total 115 mutants of *Pseudomonas corrugata* (NRRL B-30409) was tested in which two (PCM-56 and PCM-82) were selected based on their greater phosphate solubilization ability at 21°C in Pikovskaya's broth. These two mutants were found to be more efficient than wild-type strain for phosphate solubilization activity across a range of temperature, from psychotropic (4°C) to mesophilic (28°C), in aerated GPS medium containing insoluble rock phosphate with organic acid production and positive effect on all the growth parameter and soil enzymatic activity under greenhouse trial [134]. Recently, it was shown that highly conserved Asp-204 and Gly-776 are important for activity of the quinoprotein glucose dehydrogenase of *Escherichia coli* and for Mineral Phosphate Solubilization [135].

The transconjugation and expression of *pqq* genes in *Azospirillum* sp. was studied using the construct pMCG 898. The pMCG 898 containing *pqq* gene/s was mobilized into an *Azospirillum* strain negative to mineral phosphate solubilization by biparental mating. It was able to solubilize dicalcium phosphate while the wild type was not able to do so [136]. A 2.4 kb glucose dehydrogenase gene (*gcd*) of *Enterobacter asburiae,* sharing extensive homology to the *gcd* of other enterobacteriaceae members, was cloned in a PCR-based directional genome walking approach and the expression confirmed in *Escherichia coli* YU423 on both MacConkey glucose agar and hydroxyapatite (HAP) containing media. Mineral phosphate solubilization by the cloned *E. asburiae gcd* was confirmed by the release of significant amount of phosphate in HAP containing liquid medium. *gcd* was over expressed in *E. coli* AT15 (*gcd::cm*) and the purified recombinant protein had a high affinity to glucose, and oxidized galactose and maltose with lower affinities.

The enzyme was highly sensitive to heat and EDTA, and belonged to Type I, similar to GDH of *E. coli* [137].

Exploitating the Microbes in P Nutrition

The interaction between MPS microbes and plants is expected to be of synergistic nature as the MPS microbes direct the release of Pi for plant uptake and the plants provide the sugars [57] and root exudates that help the microbes for their growth. The phosphate solubilizing microbes also facilitate growth through other mechanisms [138-140, 35] as shown in Fig. 2. So, the MPS microbes are useful either singly or in consortia as bioinoculants for promoting plant growth whilst keeping the soil-health intact.

Efficient and economic use of P-fertilizers could be achieved by using phosphate solubilizing microorganisms in legumes, cereals and other useful crops. Beneficial effects of the inoculation with PSM to many crop plants have been described by numerous authors [50, 51, 141, 142]. Dry matter production, P uptake and P content were augmented significantly by the application of PSMs in many legume plants even under temperate conditions, where low temperature can restrain the microbial growth [143,144]. Increased yield to the tone of 12-15% and replacement of 25-28% of phosphate fertilizers was observed in cereals, legumes, potatoes and other field crops on the addition of rock phosphate and inoculation with PSMs [145].

Use of PSMs can increase crop yields up to 70 percent [146]. Combined inoculation of arbuscular mycorrhiza and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock [147-148]. Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixation [149]. *Pseudomonas* spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop [152]. Phosphate solubilizing bacteria enhanced the seedling length of *Cicer arietinum* [151], while co-inoculation of PSM and PGPR reduced P application by 50% without affecting corn yield [152]. Inoculation with PSB increased sugarcane yield by 12.6 percent [153]. Sole application of bacteria increased the biological yield, while the application of the same bacteria along with mycorrhizae achieved the maximum grain weight [154]. Single and dual inoculation along with P fertilizer was 30-40 % better than P fertilizer alone for improving grain yield of wheat. Dual inoculation without P fertilizer improved grain yield up to 20% against sole P fertilization [155]. Mycorrhiza along with *Pseudomonas putida* increased leaf chlorophyll content in barley [156]. Growth and phosphorus content in two alpine Carex species increased by inoculation with *Pseudomonas fortinii* [157]. Integration of half dose of NP fertilizer with biofertilizer gives crop yield as with full rate of fertilizer; and through reduced use of fertilizers the production cost is minimized and the net return maximized [158]. The inoculation of PSB (*Bacillus megaterium*) along with potential N-fixer (*Azotobacter* sp.) was found to induce resistance/ tolerance against harmful effects of salinity (ranging from 3000 to 9000 ppm) besides significantly improving growth and yield attributing parameters in wheat [159]. Thus, as revealed by several investigations, phosphate solubilizing bacteria could

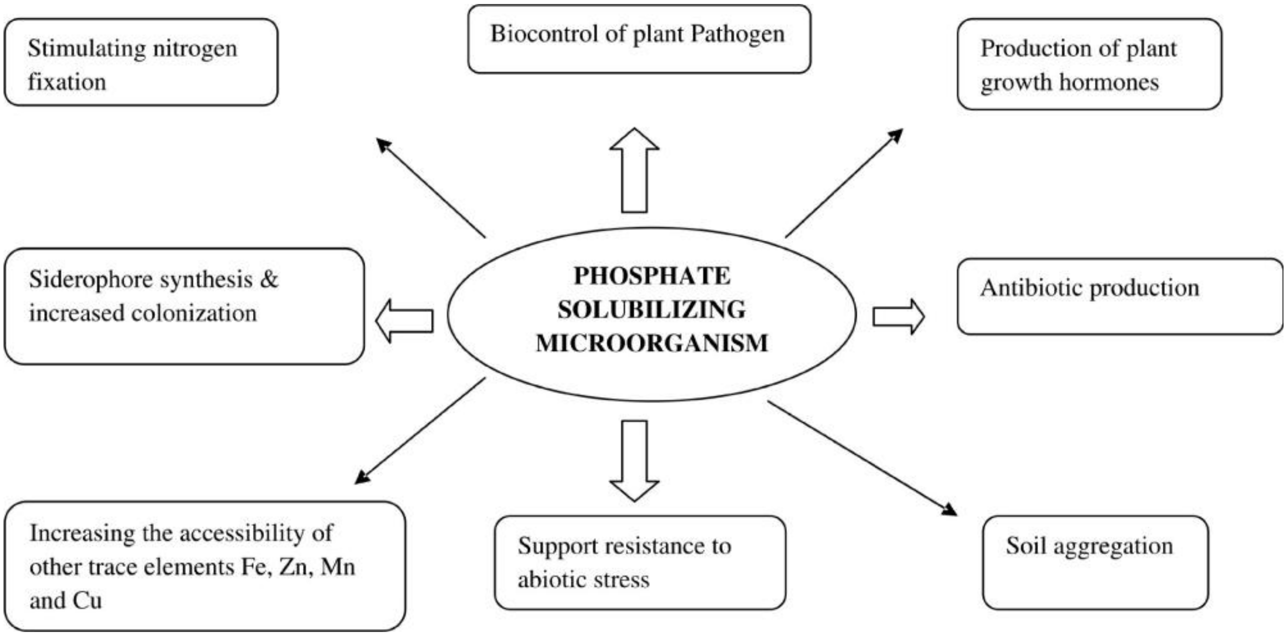

Fig. 2: Role of phosphate solubilizing bacteria in plant growth and development

increase growth and yield in several crops including walnut [159], apple [160], maize [161], soybean [162], sugar beet [163], chickpea [164,165], peanut [166], rice, tomato [167] and wheat [168].

Conclusion

The efficiency of phosphatic fertilizers is very low due to their fixation in both acidic and alkaline soils. Soils of both types predominate in India. Additionally, high acreage of saline and sodic soils also exists. Therefore, the inoculation of mineral phosphate solubilizers and other useful microbial inoculants in these soils will be important to restore the overall balance of nutrients and health of the soil to sustain it for posterity. Hence, the isolation of superior strains of mineral phosphate solubilizers to suit different soil types is imperative. Characterization of such isolates for other ancillary plant growth promoting characters is required to allow for selection of a strain with many beneficial functions or to develop consortia of compatible strains with several benefits. Engineering the soil with addition of such strains along with enough organic matter supplementation is of paramount importance to maintain the life in soil. Thus, having a strain with multiple benefits will be a better choice as inoculant since they can perform other functions as well. Thermo-tolerant multi-functional phosphate-solubilizing microbes capable of surviving the composting temperature may be useful for enrichment of compost during the process. Some of these microbes may have additional properties to the decomposition of agricultural wastes.

While the selection of the MPS strains is important, the commercial formulations also should maintain high levels of quality in terms of populations actually present in the formulation and high shelf life. Addition of high quality MPS strains can play an important role, particularly in making the direct use of abundantly available lowgrade phosphate possible. Thus, research work should not only be directed towards selection of quality MPS microbes suitable for various soil types but also should aim at identification/selection of efficient environment specific (high temperature, water deficient soils) strains for effective solubilization. Strains capable of efficient MPS activity under high buffering and high Pi levels should be isolated and tested. Engineering the microbes for higher MPS activity is an area that needs attention while simultaneously raising the issue of the acceptability of such strains for field release. Mutation through chemical mutagenesis is still a strong tool to develop more efficient MPS strains. While answering whether the MPS is a strong phenotype with distinct genetic controls has not been very effective as such, the information on the genetics and regulation of the MPS phenotype needs to be generated. Metagenomic analysis aimed at the genes involved in synthesis and release of the acids is a lean area of research and may answer the analysis of the MPS phenomenon on a community basis. At present, the production of liquid bio-fertilizers is supposed to be the breakthrough in biofertilizer technology over conventional carrier based BF-technology as liquid bio-fertilizers share more advantages like longer shelf life, constant high cell count, high enzymatic efficiency, and greater potential to fight with native population and resistance to abiotic stresses over carrier based bio-fertilizers. Therefore, special liquid formulations of PSMs should also find greater acceptance by farmers. An overall look into the various aspects of the MPS phenomenon in a concerted manner will enable its acceptance by the farming community and help the cause of clean agriculture.

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