Soil Phosphorus Fixation Chemistry and Role of Phosphate Solubilizing Bacteria in Enhancing its Efficiency for Sustainable Cropping - A review

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Phosphorus is one of the least available mineral nutrients to the plants in many cropping environments. Sub-optimal P nutrition can lead to yield losses in the range of 10% to 15% of the maximal yields. P deficiency is more critical in highly withered soils as well as in calcareous and alkaline soils. Amelioration attempts by addition of phosphatic fertilizers are economically and ecologically unsound as the efficiency of added phosphatic fertilizers is very low. Inoculation with the mineral phosphate solubilizing microbes would be reliable due to consistent performance of the inoculants under field conditions, their use also induces resistance against salinity and pathogens. Phosphorus fixation predominates in both acidic and alkaline soils, resulting in its low efficiency. Use of phosphate solubilizing microorganisms play vital role in solubilizing the insoluble forms of phosphorus. Strains from genera *Pseudomonas, Bacillus, Rhizobium, Aspergillus and Cephalosporium* are among the phosphate solubilizers. Principal mechanism for mineral phosphate solubilization involves production of organic acids, and acid phosphatases play major role in mineralization of organic phosphorous in soil. The present study highlights various forms of soil-phosphorus, phosphorus-fixation mechanism in acidic and alkaline soils and role of Phosphate solubilizing microorganisms in solubilization of fixed-phosphorus in relation to crop responses.

Key words: Phosphorus, P-cycle, Phosphorus solubilization and Soil Phosphorus fixation.

Phosphorus, the master key element is known to be involved in a plethora of functions in the plant growth and metabolism. The cellular machinery is difficult to be imagined without phosphorus being involved in its metabolic continuity and even perpetuation. Such key functions include cell division and development, photosynthesis, breakdown of sugars, energy transfer, nutrient transfer within the plant and expression. The phosphorus is present at levels of 400-1200 mg kg^{-1} of soil[1]. Its cycle in the biosphere can be described as 'open' or 'sedimentary', because there is no interchange with the atmosphere. 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 to phosphate. The genetic and biochemical mechanisms of these transformations are not yet completely understood[2]. Phosphorus in soils is present in pools varying in availability, and pools with the lowest availability are the largest in Oxisols. The link between the organic and inorganic P cycle is traditionally conceptualized as occurring only through the soil solution phase. Total P in the A and B horizons of

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an Oxisol were about 270 mg kg^{-1} [3] but the distribution of P among pools was different. For plants, readily available Pi (inorganic P, extracted by Resin and NaHCO$_3$) and the moderately available Pi (extracted by NaOH), constituted 18% of all P in the A horizon and 14% in the B horizon, whereas, the recalcitrant Pi pool (Pi extracted by concentrated HCl) was 49 and 67%, with the inert Pi (extracted by H$_2$SO$_4$–H$_2$O$_2$) constituting 9 and 14% in the A horizon and B horizon, respectively. The readily and moderately labile Po (organic P) extracted by NaHCO3 and NaOH was 19% in the A horizon and 5% in the B horizon. The Po extracted by HCl, which can include the fraction occluded in the organic matter was 5% in the A horizon and none in the B horizon. The differences in P availability and organic pools between the A and B horizon show the influence of soil biota in P transformations. These differences demonstrate how much P, which would end up in fractions that are very hard to recover, has been (and could further be?) mobilized through plants and microorganism.

Phosphate forms available in soil

In soil P exists chiefly as orthophosphate although phosphine and phosphonates have been detected under some conditions. The diverse P forms can be categorized as soil solution p, insoluble inorganic P and insoluble organic P. Only small fraction of soil P exist in soil solution (1 ppm or 0.1%), is readily available to plants because of its low solubility and fixation in soils. The cell might take up several forms of P but the greatest part is absorbed in the forms of H$_2$PO$_4$-, HPO$_4^{2-}$ depending up on soil pH largely.   Mineral phosphate can be also found associated with the surface of hydrated oxides of Fe, Al, and Mn, which are poorly soluble and assimilable (insoluble inorganic P). 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. Most agricultural soils contain large reserves of phosphorus, a considerable part of which has accumulated as a consequence of regular applications of P fertilizers[4]. 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. 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[5]. Superphosphate contains a sufficient amount of calcium to precipitate half of its own P, in the form of dicalcium phosphate or dicalcium phosphate dihydrated[6].
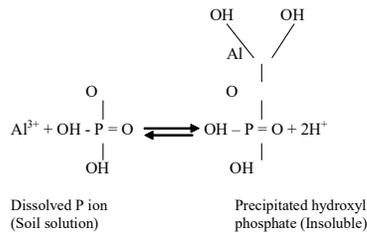
A third major component of soil P is in organic. Organic forms of P may constitute 30-50% of the total phosphorus in most soils, although it may range from as low as 5% to as high as 95%. Organic P in soil is largely in the form of inositol phosphate (soil phytate). It is synthesized by microorganisms 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. 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. 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 occurs in soil, although values as high as 34 ppm has been detected. 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$_2$PO$_4^{-}$), or low molecularweight organic phosphate, to be assimilated by the cell[7]. 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[8]. The biggest reserves of phosphorus are rocks and other deposits, such as primary apatites and other primary minerals formed during the geological age. For example, it is estimated that

there is almost 40 million tons of phosphatic rock deposits in India and this material should provide a cheap source of phosphate fertilizer for crop production[9]. 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 by microbial actions.
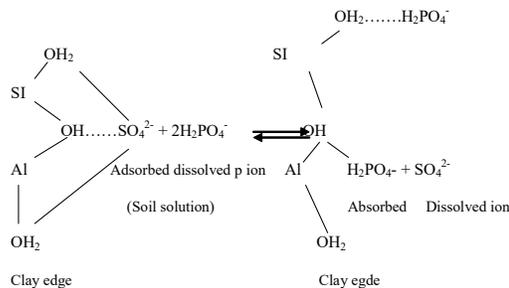
Phosphate fixation (The mechanism which restrains P availability and efficiency)

The term phosphorus fixation refers the

fixation of inorganic-P by Al, Fe oxides and Ca to produce P containing compounds of lower solubility, when P fertilizers are added to soils. It involves series of reactions which removes P from soil solution render it unavailable for plants and reduces its efficiency to only 15-20% temporary or permanently depending upon stage/time of chemical fixation reactions.

P-Fixation in acidic soils

Most of the P-fixation occurs in acidic soils, where $H_2PO_4^-$ reacts with the surfaces of insoluble oxides of iron, almonium and manganese, involves series of chemical fixation reactions and thus interlocks the P. Some of these reactions are given as under:

A) Precipitation reaction

C. Reaction with Al or Fe oxide surfaces (inner sphere)

Hydrous oxide surfaces

B. Anion exchange reaction (outer sphere)

D) Formation of stable binuclear bridge (inner sphere)
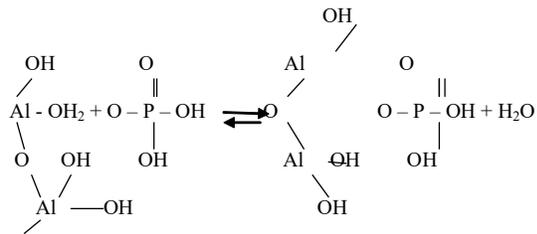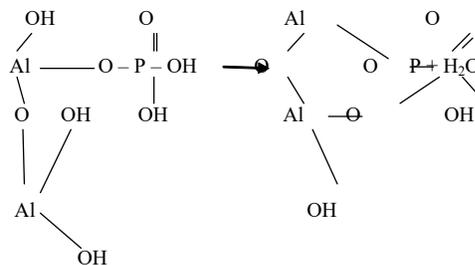

Summary of the P-fixation reactions

In reaction (A), freshly formed hydroxyl phosphate is slightly soluble, because of having a greater surface area exposed to the soil solution. Therefore, P in it is available initially to some extent to the plants. With advanced time, the precipitated hydroxyl phosphate ages and become less soluble and becomes totally unavailable for plants. In reaction (B), phosphate is reversibly adsorbed by anion exchange with broken clay edges of kaolinite

clays. In reaction (C), the phosphate ion replaces –OH group in the surface structure of Al oxide minerals and in reaction (D), the phosphate further penetrates the mineral surface by forming a stable binuclear bridge.

P-fixation in alkaline soils

The availability of P in alkaline soils is determined principally by the solubility of the various Ca-phosphate compounds present. In alkaline soils, soluble H_2PO_4 quickly reacts with

calcium to form a sequence of products of decreasing solubility. For instance, highly soluble mono-calcium phosphate $(CaH_2PO_4)4$ added as concentrated superphosphate fertilizer rapidly reacts with Calcium carbonate $(CaCO_3)$ to form first dicalcium phosphate and then reacts again with $CaCO_3$ to form tri-calcium phosphate with decreasing solubility gradually. The tri-calcium phosphate may undergo further reactions to form even more insoluble compounds, such as, hydroxyl carbonates and hydroxyl apatites. These compounds are thousand times less soluble than freshly formed tri-calcium phosphates. The extreme insolubility of apatites in neutral or alkaline soils generally makes powdered phosphate rocks (which consists mainly of apatite minerals), not very effective source of P for plants unless it grounded to very fine to increase weathering surface and applied to relatively acidic soils.

$$CaH_4(PO_4)2 + CaCO_3 \rightleftharpoons Ca_2H_2(PO_4)_2 + CO_2 + H_2O$$
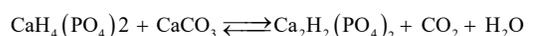
Monocalcium-P   Ca-carbonate   Dicalcium Phosphate (Less soluble)

$$Ca_2H_2(PO_4)_2 + CaCO_3 \rightleftharpoons Ca_3(PO_4)_2 + CO_2 + H_2O$$
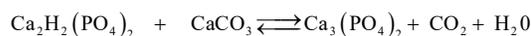
Tricalcium phosphate (Least soluble)
Tricalcium phosphate → Carbonate and Hydroxy apatite (Extremely insoluble)

Role of P solubilizing bacteria in P solubilization

The introduction of efficient microbes (P-solubilizers) in the rhizosphere has been found to increase the availability of phosphorus from both applied and native soil phosphorus. The microbial property of dissolving interlocked phosphates appears to have an important implication in Indian agriculture and same has been documented by many investigators[10,11,12,13]. Primarily there are two schools following of thought interpreting the mechanism of P-solubilization by microorganisms (Phosphate solubilizers)[12].

  Solubilization by production of organic acid
  Solubilization by production of phosphatase enzymes

Mineral phosphate solubilization by organic acids (Organic acids and P Solubilization)

Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as almonium and iron bound phosphate, tricalcium phosphate, dicalcium phosphate, hydroxyapatite,

and rock phosphate. Among the bacterial genera with this capacity are *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aereobacter*, *Flavobacterium*, *Erwinia* and among the fungi are *Aspergillus sp.*, *A. awamon*, *Cephalosporium sp.*, *P. balagi*, *Rhizopous sp.*, *Alternaria sp.* etc. There are considerable populations of phosphate-solubilizing bacteria/fungi in soil and in plant rhizospheres. These include both aerobic and anaerobic strains, with a prevalence of aerobic strains in submerged soils. A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizoplane and rhizosphere in comparison with non rhizosphere soil[14]. The soil bacteria belonging to the genera *Pseudomonas* and *Bacillus* and Fungi are more common. The major microbiological means by which insoluble-P compounds are mobilized is by the production of organic acids, accompanied by acidification of the medium. The organic and inorganic acids convert tricalcium phosphate to di and monobasic phosphates with the net result of an enhanced availability of the element to the plant.

Table 1. Interaction effect of Rhizobium, PSB and fertility levels on seed and straw yield of Green gram under temperate conditions of Kashmir

Treatment	Seed yield (q ha^{-1})	Straw yield (q ha^{-1})
T1 N0 P0 Rh0 PSB0	6.38	16.53
T2 N0 P0 Rh1 PSB0	6.41	16.56
T3 N0 P0 Rh0 PSB1	6.40	16.55
T4 N0 P0 Rh1 PSB1	6.46	17.11
T5 N10 P30 Rh0 PSB0	8.47	21.56
T6 N10 P30 Rh1 PSB0	9.55	23.90
T7 N10 P30 Rh0 PSB1	9.04	22.80
T8 N10 P30 Rh1 PSB1	9.78	24.39
T9 N20 P45 Rh0 PSB0	9.72	24.38
T10 N20 P45 Rh1 PSB0	11.49	28.51
T11 N20 P45 Rh0 PSB1	11.23	27.96
T12 N20 P45 Rh1 PSB1	11.82	28.98
T13 N30 P60 Rh0 PSB0	10.18	25.66
T14 N30 P60 Rh1 PSB0	11.35	28.32
T15 N30 P60 Rh0 PSB1	11.21	28.02
T16 N30 P60 Rh1 PSB1	11.57	28.81
SEm±	0.20	0.38
CD (P=0.05)	0.59	1.11

*N= Nitrogen; P= Phosphorus; Rh0 and PSB0= Uninoculated; Rh1 and PSB1=Inoculated

The type of organic acid produced and their amounts differ with different organisms. Tri and di-carboxylic acids are more effective as compared to mono basic and aromatic acids. Aliphatic acids are also found to be more effective in P-solubilization compared to phenolic, citric and fumaric acids. The analysis of culture filtrates of PSMs has shown the presence of number of organic acids including citric, fumaric, lactic, 2-ketogluconic, gluconic, glyoxylic and ketobutyric acids. These acids may also compete for fixation sites of Al and Fe insoluble oxides, on reacting with them, these acids stabilize them and are called 'chelates'. The extent of P-solubilization also depends on the accessory minerals present in the rock phosphate. The solubilized phosphate may react with Ca and Mg present in the rock phosphate as soon as pH of the growth medium increases. Presence of free carbonates in rock phosphates also reduces the extent of solubilization as a large part of organic acids is directed towards neutralization of free carbonates.

Organic phosphate solubilization by phosphatase enzymes (Enzymes and P Solubilization)

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. The microbial mineralization of organic phosphorus is strongly influenced by environmental parameters; in fact, moderate alkalinity favors the mineralization of organic phosphorus. 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. 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. In a recent published comprehensive review of bacterial nonspecific acid phosphohydrolases. The specific phosphohydrolases with different activities include: 39- nucleotidases and 59-nucleotidases; hexose phosphatases; and phytases[15]. A specific group of P releasing enzymes is those able to cleave C-P bonds from organophosphonates[16]. 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

Table 2. Mean values of number of spikes and weight of 1000 kernel of wheat as affected by different Phosphate solubilizing microorganism treatments under different salinity levels

	Salinity levels (ppm) Plant age (days)									
	1st sample (45 Days) No. of spikes plant⁻¹					2nd sample (105 Days) Wt. of 1000 kernel				
Biofertilizer	0	3000	6000	9000	Mean	0	3000	6000	9000	Mean
Control	4.51	3.30	2.17	0.82	2.70	18.85	16.46	12.18	6.17	13.41
Cerealien (Rhizobium)	6.27	4.61	4.24	2.55	4.42	25.49	20.19	18.24	11.06	18.75
Phosphorine (PSB)	6.20	4.70	3.52	2.74	4.29	24.50	21.05	17.50	11.52	18.64
Cerealien + Phosphorine	8.10	7.15	5.67	4.11	4.62	29.23	25.21	22.14	18.65	23.81
Mean	6.29	4.92	3.86	2.60		24.52	20.73	17.51	11.85	
LSD value at 0.05 Salinity	0.26			0.49						
Bio-fertilizers	0.21			0.49		Salinity x Biofertilizer 1.12				

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.

Effect of PSMs on growth, yield and P economy

Efficient and economic use of P-fertilizers could be achieved by using phosphate solubilizing microorganisms in legumes, cereals and other useful crops. 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 [10, 17]. 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[12]. Seed and straw yield of Green gram obtained with 45kg P_2O_5 ha^{-1} + PSB was found

statistically *at par* with the yields obtained with 60 kg P_2O_5 ha^{-1} with or without inoculation under temperate conditions of Kashmir (Table 1) of north India[11]. The inoculation of PSB (*Bacillus megaterium*) along with potential N-fixer (*Azotobacter sp.*) were found to induce resistance/tolerance against harmful effects salinity (ranging from 3000-9000 ppm) besides improving growth and yield attributing parameters significantly (Table 2) in wheat[18]. Under subtropical conditions of Uttar Pradesh, it is concluded that the inoculation of PSB + 45 kg P_2O_5 (B_2P_3) treatment in wheat recorded highest grain yield and proved significantly better than any other interaction under test[13] (Table 3). The fact can be attributed due to high P-uptake by plant with due to increased solubility and mobility of soil phosphorus, which in turn has increased the root morphological attributes and thereby also inducing the drought resistance to the plant. PSB + VA-mycorrhiza inoculations were found to reduce the damage caused by soil-borne pathogenic fungi, nematodes and bacteria in crops by increasing the solubility, mobility and uptake of soil phosphorus as plants with good phosphorus status are less sensitive to pathogen damage[19].

Table 3. Interaction effect of PSB, VA-mycorrhiza and phosphorus on
grain yield of wheat under subtropical conditions of U.P.

Treatment	Levels of Phosphorus (Kg ha^{-1})				
	P1 (15)	P2 (30)	P3 (45)	P4 (60)	Mean
B1 (Control)	30.16	36.33	41.50	45.00	38.25
B2 (VAM)	32.16	38.33	46.66	45.00	40.54
B3 (PSB)	39.16	45.50	50.33	49.01	46.00
Mean	33.83	40.05	46.16	46.33	
SEm±	1.78				
CD (P=0.05)	3.75				

CONCLUSION

The efficiency of phosphatic fertilizers is very low (15-20%) due to its fixation in acidic and alkaline soils and unfortunately both soil types are predominating in India accounting more than 34% acidity affected and more than seven million hectares of productive land salinity/alkalinity affected. Therefore, the inoculations with PSB and

other useful microbial inoculants in these soils become mandatory to restore and maintain the effective microbial populations for solubilization of chemically fixed phosphorus and availability of other macro and micronutrients to harvest good sustainable yield of various crops. Commercial exploitation of phosphatic microbial inoculants can play an important role particularly in making the direct use of abundantly available low grade

phosphate possible. Research work should be extended for identification/selection of efficient location specific strains for effective Psolubilization. Those P-solubilizing organisms derived from rhizoplane have the highest capacity of solubilization than those derived from rhizosphere and non-rhizosphere. At present the production of liquid bio-fertilizers is supposed to be the breakthrough in biofertilizers 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 coupled with genetically enhanced plants with better P use efficiency (PUE) through efficient P absorption, transportation and internal utilization should also find greater acceptance by farmers.

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