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Phosphate-solubilising Microorganisms as Potential Biofertilizer: A Review

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ABSTRACT

After the nitrogen phosphorus is the second most important plant nutrient to necessary for plant development and growth. The use of excess phosphate fertilizers potentially causes surface and ground water pollutions and soil fertility depletation and accumulation of phosphate in soil which is unavailable for plant. Biofertilizers play a very significant role in improving soil fertility by fixing atmospheric nitrogen, both, in association with plant roots and without it, solubilized insoluble soil phosphates and produces plant growth substances in the soil. There have been a number of reports on plant growth promotion by microorganisms that have the ability to solubilize inorganic and organic P from soil. There is a dynamic and complex relationship among the different forms of P involving soil, plant and microorganisms. Microorganisms can enhance the capacity of plants to acquire P from soil through various mechanisms. They are able to solubilize unavailable form of unsolubilized phosphate in available form. Purpose of this review is to focuses on the understanding of the mechanism of phosphate solubilisation their role of PSMs (phosphate solubilizing microorganisms) in crop production as biofertilizers.

Key words: Microorganisms, Phosphate solubilizer.

Phosphorus (P) exists in soil in organic and inorganic forms. Each form is a continuum of many P compounds, existing in different phases and in equilibrium with each other. Availability of P ranges from soluble P (plant available) to very stable (plant unavailable) compounds (Fig 1). There is a dynamic and complex relationship among the different forms of P involving soil, plants and microorganisms.

Organic P in compounds is found in humus and other organic material including decayed plants, animal and microbial cells. Phosphorus is labile organic compounds, can be slowly mineralized or broken down and released as available inorganic phosphate. The process of mineralization or immobilization is carried out by microorganisms and is highly influenced by soil moisture and temperature.

Phosphate availability in soil

Phosphorus is one of the major essential macronutrients for biological growth and development. It is present at level of 400-1200 mg kg^{-1} of soil (Fernandez *et al.,* 1988). Its cycle in the biosphere can be described as 'open' or sedimentary' because there is no inter-change with the atmosphere (Begon and Harper, 1990). Phosphorus in fertilizers is converted to water soluble P as orthophosphate ions $H_2PO_4^-$ and HPO_4^{2-} in soil, within a few hours after application (Schulte and Kelling, 1996). In most soils, orthophosphate ions $H_2PO_4^-$ and HPO_4^{2-} dominate at pH below 7 and above 7.2, respectively (Hinsinger, 2001). In most soils, maximum P availability occurs between pH 5.5 to 7, within this pH range, P is fixed by hydrous oxides of Fe, Al and Mn. Between pH 6 to 8 and pH 6.5 to 8.5 phosphate is fixed by silicate minerals and Ca, respectively. As a result, the most efficient use of P in neutral and calcareous soils occurs between pH 6 to 7 (Sharpley, 2006). Phosphate fertilizers can increase P availability initially, but will promote the formation of insoluble P minerals and consequently lead to P builds up. Therefore, P management is important both environmentally and economically. Phosphate solubilizing microorganisms may be an answer for maintaining the supply of plant available P because PSM carry out the conversion from labile P to plant available P (Sharpley, 2006).

Phosphate solubilizing microorganisms

Phosphate solubilizing microorganism plays a central role in the natural phosphorus cycle. Phosphate solubilizing microorganism (PSM) include largely bacteria and fungi, which can grow in media containing tricalcium, iron and aluminium phosphate, hydroxyapatite, bone meal, rock phosphate and similar insoluble phosphate compounds as the sole phosphate source. Such microbes not only assimilate P but a large portion of soluble phosphate is released in the environment (Gaur, 1990).

The most efficient PSM belong to the species of *Bacillus,Streptomyces, Aspergillus* and *Penicillium*.

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The reported bacilli include *B. brevis, B. cereus, B. licheniformis, B. megaterium, B. polymyxa, B. pulvifaciens* and *B. subtalis* from the rhizosphere of legumes, cereals, oat, arecanut, palm, jute and chilli (Bhattacharya, 1998; Gaur, 1999). *Pseudomonas striata, P. cissicola, P. fluorescens, P. putida, P. syringae, P. putrefaciens* and *P. sturzeri* have been isolated from rhizosphere of *Brassica*, chick pea, maize, soybean and other crops. Cyanobacteria like *Anabaena, Calothrix, Nostoc, Scytonema* and *Ceylonica* also reported for solubilizing phosphates (Gupta and Vyas, 1998).

Among phosphate solubilizing fungi, *Aspergillus niger*, *A. flavus, A. nidulans, A. awamori, A. carbonum, A. fumigatus, A. terreus* and *A. wentii* have been reported from the rhizosphere of maize, soybean, chilli, tista soils, acidic lateritic soil and compost (Prerna and Kapoor, 1997). *Jumpstart (R*) is the first P-solubilizing inoculants available in the market and the active ingredient is the fungus *Penicillium bilaiae* formerly known as *Penicillium bilaii. P. bilaiae* is known for its superior ability in Ca-P solubilization (Kucey, 1988).

Among soil born microorganisms, actinomycetes are of special interest since they possess many properties that could benefit the plant growth and fitness (Errakhi *et al.*, 2007). These bacteria strongly adhere to the soil particles and establish intimate contact (endophytic property) with plants (Conn *et al.*, 2008). Many strains of actinomycetes also have the protective effect against phyto-pathogenic fungi (Hamdali *et al.*, 2008). The endophytic properties of actinobacteria, other than *Frankia* (De Araujo *et al.,* 2000) have previously been reported for *Streptomyces griseus, Streptosporangiaceae*strainPA147*, Streptomyces argenteolus, Streptomyces peucetius* and *Nocardioides albus* (Coombs and Franco 2003). Actinomycetes strains are thought to greatly improve the efficiency of the delivery of nutrient from the microorganism to the plant (Firakova *et al.*, 2007).

Mechanism of phosphate solubilization

Mineral phosphate solubilization

Several theories exist explaining the mechanism of microbial phosphate solubilization. The sink theory (Halvorson *et al.*, 1990), the organic acid theory (Cunningham and Kuiack, 1992) and the acidification by H^+ excretion theory (Illmer and Schinner, 1995) have been well explained. In sink theory, P solubilizing organisms are able to remove and assimilate P from the liquid and therefore stimulate the indirect dissolution of Ca-P compounds by continuous removal of P from broth (Halvorson *et al.*, 1990).

Major mechanism of mineral phosphate solubilization is the action of organic acids production by microorganisms. The insoluble sources of inorganic phosphorus (P) in liquid broth are solubilized by phosphate solubilizing microorganisms accompanied by the production of organic acids. The action of organic acids synthesis and lowering the pH cause dissolution of P compounds (Whitelaw, 2000; Pradhanand Sukla, 2005). The production of organic acid leads to acidification of microbial cells and their surroundings and, consequently, the release of P ions from the P mineral by H^+ substitution for Ca^{2+} (Goldstein, 1994). Various organic acids are identified by the liquid cultures of PSM and can be associated with specific microbial groups *e.g.* 2-ketogluconic acid and oxalic acid are commonly found in bacterial and fungal cultures, respectively. Gluconic, acetic and lactic acids have been observed from both types of microorganisms and gluconic acid seems to be the principal organic acid frequently found among PSM. There is also experimental evidence that supports the role of organic acids in mineral phosphate solubilization. Halader *et al.,* (1990) showed that the organic acids isolated from a culture of *Rhizobium leguminosarum* solubilize an amount of P nearly equivalent to the amount that has been solubilized by the whole culture. Besides this, treatment of the culture filtrate from several *Rhizobium* strains with pepsin or removal of protein by acetone precipitation do not affect phosphate release capacity showing that this is not an enzymatic process. Goldstein (1994) proposed that the direct periplasmic oxidation of glucose to gluconic acid and 2-ketogluconic acid forms the metabolic basis of mineral P solubilization in some gram negative bacteria.

Lack of linear correlation between pH and the amount of P solubilization suggested some alternative possibilities other than organic acid production (Asea *et al.,* 1988). In addition, no significant amount of organic acid production could be detected in a phosphate solubilizer fungus, *Penicillium* sp. (Illmer and Schinner, 1992). Illmer and Schinner (1995) suggested H^+ excretion theory to explain Ca-P solubilization accompanied by a decrease in pH. They observed that the release of H^+ to the outer surface in exchange for cation uptake or with the help of H^+ translocation ATPase could constitute alternative ways for solubilization of mineral phosphate.Other mechanisms of phosphate solubilization such as the production of chelating substances by PSM either by lowering the pH or by enhancing chelation of the cations bound to P chelation involves the formation of two or more co-ordinate bonds between a molecules (the “ligand”) and a metal ion resulting in a ring structure complex. Chelation by an organic acid ligand occurs *via* oxygen contained in hydroxyl and carboxyl groups (Whitelaw, 2000).

Organic phosphate 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. Organic phosphorus cannot be utilized by plants without their microbial conversion to inorganic form bacteria, fungi and actinomycetes by their enzymatic action make the bound organic phosphates available to plants.

Mineralization is also favoured by warm temperature, the thermophilic range being more favourable than the mesophilic range. The rate of mineralization is augmented by a shift in the pH from acidity to neutrality. The rate of

mineralization in generally correlated to the quantity of the substrates. Hence, soils rich in organic phosphates will be most active. The mineralization of organic phosphorus is not inhibited by inorganic phosphates (Daughtrey *et al.*, 1973). The enzymes which cleave phosphorus from organic substrates are known as phosphatase (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.

Some phosphophydrolases are secreted outside the plasma membrane, where they are either released in a soluble form or retained as membrane bound proteins. This localization allows them to act as scavenging enzymes on organic phosphoesters that are components of high molecular weight materials (*i.e.* RNA and DNA) and cannot cross the cytoplasmic membrane. This material can be first converted to low molecular weight compounds and this process may occurs 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 phosphohydrolase providing the cell with essential nutrients (Goldstein, 1994).

Phosphate solubilizing microorganism as inoculants

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 predominated in India accounting more than 34% acidity affected and more than seven million hectares productive land salinity affected. Therefore, the inoculations with PSM and other useful microbial inoculants in these soils became 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 (Mahdi and Hassan, 2011). There have been a number of reports on plant growth promotion by microorganisms that have the ability to solubilized inorganic and organic P from soil. Several phosphate solubilizing microorganism occur in soil, but the amount of P liberated by them is generally not sufficient for a substantial increase in *in situ* plant growth therefore, inoculation of plants by a target microorganism at a much higher concentration than that normally found in soil is necessary to take advantage of the property of phosphate solubilization for plant yield enhancement (Rodriguez and Fraga, 1999).

Many workers in India and elsewhere have studied the effect of phosphate solubilizing microorganisms have reported the increase in yield of various crops (Rao, 1986, Hegde *et al.*, 1998; Biswas *et al.*, 2001). Biswas *et al.*, (2001) observed the contribution of bio-fertilizers supplying 36kg of N/ha and 22.6 kg of P_2O_5/ha in wheat crop beside economic benefit of Rs. 2,059/ha. They also reported the increased yield of 11.9% in pearl millet, 10% in finger millet, 9.9% in sorghum, 8.9% in maize and 8.4% in barley (Biswas *et al.,* 2001).

Mittal *et al.* (2008) observed the effect of six phosphate solubilizing fungi, including two strai\ ns of *A. awamori* and

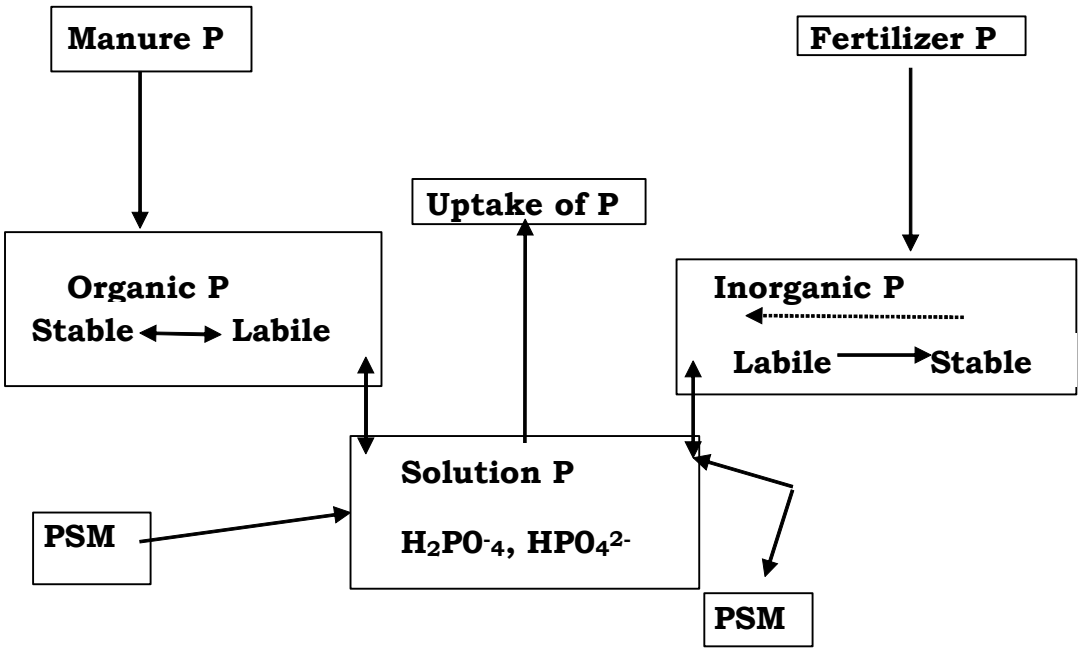


Fig 1: The soil phosphorus cycle (adapted from Sharply, 2006), solid line indicates the conversion process. The dashed line means very slow conversion.

four strains of *P. citrinum*, on growth and seed production of chickpea *(Cicer arietinum* L. Cv. GP f_2) plants in pot experiments. The various pot experiments carried out in the greenhouse, maximum stimulatory effect on chickpea plants has been observed by inoculation of two *A.awamori* strains. This treatment resulted in a 7-12% increase in shoot height, a nearly threefold increase in seed number and a two fold increase in seed weight as compared to the control (uninoculated) plants. While a consortium of all the six fungal isolates showed no stimulatory effect on chickpea plants. Similarly, the inoculation of *P. oxalicum* CBPS-3F-TS used either alone or along with fused phosphate (FP) and rock phosphate, increased the growth and N and P accumulation in maize (*Zea mays*) plants compared to the control (Shin *et al.,* 2006). Several workers have also reported a profound increase in yield of wheat (*Triticum aestivum*) (Whitelaw *et al.,* 1997) and soybean (*Glycine max*) and faba bean (*Vicia faba*) (Abd-Alla *et al*., 2001) through inoculation of P-solubilizing fungi.

Phosphate solubilizing also increase the yield of rice and wheat plants. Preplant inoculation of rice seedling roots or wheat seeds with P-solubilizing fungus *A. awamori* led to a yield increase over non-inoculated treatments by 0.09-0.22 tan ha^{-1} in rice and 0.15-0.45 tan ha^{-1} in wheat (Dwivedi *et al*., 2004). The agronomic efficiency and recovery efficiency of fertilizer P in the rice-wheat system have been highest [57.2 kg grain (kg P)$^{-1}$ and 40.4%, respectively] under diammonium phosphate fertilizer treatments. The agronomic efficiency ranged from 14.3-44.4 kg grain (kg P)$^{-1}$ and the recovery efficiency increased with increasing initial rock phosphate application and with P-solubilizing fungus inoculation. Combined inoculation of N_2 fixers and P solubilizers may benefit plants better than either group of microorganism alone.

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