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Review Article

PHOSPHATE SOLUBILIZING MICROBES: AN EFFECTIVE AND ALTERNATIVE APPROACH AS BIOFERTILIZERS

KUMAR ANAND[1], BABY KUMARI[1*], M. A. MALLICK[1]

[1]University Dept. of Biotechnology, Vinoba Bhave University, Hazaribag, Jharkhand
Email: baby.rai@gmail.com

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ABSTRACT

It is undoubtedly clear that phosphorus is the second most important nutrient after nitrogen required for growth of plants. It is an essential element in all living systems. Hardly 1%-2% of phosphorous is supplied to other parts of the plants. Plants acquire phosphorus from soil solution in the form of phosphate anion. It is the least mobile element in plant and soil in comparison to other macronutrients. It remains in a precipitated form in the soil as mono or orthophosphate or is absorbed by Fe or Al oxides through legend exchange. Generally, the phosphate solubilizing microorganisms (PSM) play a very important role in phosphorus nutrition by exchanging its availability to plants through release from inorganic and organic soil phosphorus pools by solubilization and mineralization. The main mechanism in the soil for mineral phosphate solubilization is by lowering the soil pH by the microbial production of organic acids and mineralization of organic phosphorus by acid phosphates. To fulfill the phosphorous demand of plant, an additional source of phosphorous is applied to plants in the form of chemical fertilizers. One of the most common forms of phosphate is fertilizers in the form of rock phosphate or superphosphate. It is not suggested to apply these phosphates directly to soil as there are so many environmental problems. Hence, biofertilizers or microbial inoculants are used as an alternate source, which are both economic as well as eco-friendly.

Keywords: PSM, Phosphate solubilization, Phosphatase, Economic, Eco-friendly.

INTRODUCTION

Phosphorous is a major growth limiting nutrient. As like nitrogen, there is no large atmospheric source that can be made biologically available. Phosphorous (P) plays an important role in plant growth and is the major plant growth limiting nutrient despite its abundance in soils in both inorganic and organic forms [1]. Metabolic processes (photosynthesis, energy transfer, signal transduction), Nitrogen fixation in legumes, crop quality and resistance to plant diseases are main features associated with phosphorous nutrition [2-4]. It is absorbed by the plants, in the orthophosphate (H_2PO4^- and $HPO_4^{2-)}$ forms [5]. Phosphorus being a structural component of many coenzymes, phospho-proteins, phospholipids [6] also forms a part of the genetic memory "DNA" of all living organisms. It is involved in the transfer and storage of energy which is used for growth and reproduction. Phosphorus is important in several physiological processes of plants, especially in photosynthesis, carbon metabolism, and membrane formation [7]. Also, it plays the vital role in root elongation, proliferation, and its deficiency affects root architecture [8, 9], seed development and normal crop maturity. Phosphorus is readily translocated within the plants, moving from older to younger tissues as the plant forms cells and develops roots, stems, and leaves. A major portion of phosphorus absorbed by the plant is accumulated grains as phytase [10] and its deficiency negatively affects grains yield.

Strategy needed to combat effect of P-fertilizers

Although phosphorous is an essential macronutrient for plant growth and development, 95-99% of it, present in the soil is insoluble and cannot be utilized by plants [11]. This unavailability is due to P-fixation, either it is adsorbed on the soil minerals or get precipitated by free $Al^{3+}$and Fe^{3+}in the soil solution [12, 13]. To increase the availability phosphorus to plants, a large amount of phosphorous is quickly applied to soil, a large portion of which is quickly transferred into insoluble form [14] and very little percentage of applied phosphorus is available to plants making continuous application fertilizer necessary [15]. Soil phosphorous dynamics is characterized by physicochemical and biological processes. A large amount of phosphorous applied as fertilizers enters into the immobile pools through precipitation reaction with highly reactive $Al^{3+}$and Fe^{3+}in acidic and Ca^{2+}in calcareous or normal soils [1, 16]. Organically bound phosphorous enters in the soil during the decay of natural vegetation, dead animals and from excretions. Although phosphorous it, present in the soil is insoluble and cannot be utilized by plants [11]. The changing scenario demands to adapt the strategies/approaches to convert an unavailable fraction of soil P into available form for plant uptake. Among the various strategies known for this purposes, use of specific microflora is considered as one of the most efficient means to solubilize insoluble soil P. Although microbial inoculants are in use for improving soil fertility during last century, however rarely sufficient work has been reported on Phosphate Solubilizing Microorganisms as compared to nitrogen fixation. Phosphate solubilizing microbes have the potential of making these phosphates available to the plants. Similarly, starch, as well as cellulose-degrading microbes, can degrade these complex carbohydrates and increase the soil fertility. Phosphate solubilizing bacteria (PSB) are being used as biofertilizer since the 1950s [17, 18]. There are also some evidence of naturally occurring rhizospheric phosphorus solubilizing microorganism (PSM) since 1903 [19]. Bacteria are more effective in phosphorus solubilization than fungi [20]. Among the whole microbial population in soil, Phosphate Solubilizing Bacteria (PSB) constitute 1 to 50 %, while phosphorus is solubilizing fungi (PSF) are only 0.1 to 0.5 % in P solubilization potential [21]. The bacterial strain isolated from alkaline soil can solubilize phosphates at high salt, high pH and high-temperature concentration [22, 23]. Many phosphate solubilizing bacteria are reported as plant growth promoter in many crops like tomato, rice etc [22, 24-27].

Diversity of PSM

There is a myriad of microorganisms, especially the Phosphate Solubilizing Microorganisms (PSM) present in the soil. Some of the most common examples are the species of *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Aspergillus, Penicillium, Fusarium, Sclerotium, etc.* Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi are the most powerful P solubilizer [28]. *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa, B. sircalmous, Pseudomonas striata*, and *Enterobacter* are referred as the most important strains

[29,30]**.** Commonly there are more bacilli in soil whereas spirilli are very rare in natural environments [31**.** The PSB are ubiquitous with variation in forms and population in different soils. The population of PSB depends on different soil properties (physical and chemical properties, organic matter, and P content) and cultural activities [32]**.** Larger populations of PSB are found in agricultural and rangeland soils [33]**.**

Isolation and identification of phosphate solubilizing microorganisms

To isolate, the Phosphate Solubilizing Microorganisms, Pikovaskaya's medium is prepared. It is mixed with gum arabic and autoclaved and dispensed in Petri plates. Generally a small amount of soil, approximately 1 gm of soil is taken from the field and serially diluted in the known volume of water. Each plate is inoculated with 1 ml of sterile soil water suspension. Plates are incubated at 28 for about 5 d to 7 d. Only Phosphate Solubilizing Microorganisms grow and form the colony which can be identified due to the formation of a clear zone around each colony. It is only because the microorganisms grow and utilize calcium phosphate. Such colonies are picked up, purified and preserved for further identified through biochemical and morphological identification, molecular characterization through 16 s RNA sequencing is done for identification of PSM strains [34].

According to the literature, the Pikovskaya's agar medium (PVK) was found to be as selective media for the isolation of PSM [35]. P^H is maintained at 7.0. The composition of the medium was as given in the table mentioned above (table 1).

Table 1: Composition of pikovskaya's agar medium (PVK) (Composition in gm/ml)

Glucose	**10**
Yeast Extract	0.5
$(NH_4)_2SO_4$	0.5
$MgSO_4.7H_2O$	0.1
$Ca_3(PO_4)_2$	5
NaCl	0.2
KCl	0.2
$MnSO_4.2H_2O$	0.002
$FeSO_4.7H_2O$	0.002
Agar	1.5

The phosphate solubilizing microorganisms solubilize 20%-30% phosphate which is then absorbed by plants. The PSM can be used for all types of plants because they are heterotroph and show host specificity.

Mechanism of action of PSM

The efficiency of P fertilizer throughout the world is around 10-25 % [36] and concentration of bioavailable P in soil is very low reaching the level of 1.0 mg kg–1 soil [37] Plants can absorb phosphate only in soluble form. The transformation of insoluble phosphate into soluble form is performed by a. Soil microorganisms play a key role in soil P dynamics and subsequent availability of phosphate to plants [38]. Phosphate Solubilizing Microorganisms (PSMs) especially Phosphate Solubilizing Bacteria (PSB) enhance the solubilization of insoluble phosphorous compounds through the release of organic acids and phosphatase and phytase enzymes[4] which is present in a wide variety of soil microorganisms. Species such as *Pseudomonas, Mycobacterium, Micrococcus and Flavobacterium* among bacteria and *Penicillium, Sclerotium, Aspergillus* and many other fungi are active in the conversion. During the conversion process, a part of phosphorous is assimilated by microorganisms, but the amount made soluble and released is in excess to the requirement of the microorganisms. The excess amount thus released is made available to plants. During this conversion process, organic acids play an important role. Equally important are nitric acid and sulphuric acid. As a result, these organic and inorganic acids convert calcium phosphate to di or monobasic phosphates and are then easily made available to plants phosphates [19, 39-41]. The amount of carbohydrates being oxidized by heterotrophs has a great impact upon the solubilization process Nitric and sulphuric acid are produced by the oxidation of nitrogenous materials or inorganic compounds of sulfur, act upon rock phosphate [34]**.** During this process phosphate, solubilization is affected by nitrification of ammonium salts. Generally, the solubilization of phosphate is achieved by acid production [2, 3]. Mobilization of ferric phosphate requires certain bacteria which liberate hydrogen sulfide. A number of phosphate dissolving microorganisms are also known well in soil. It is also known that root region is abundantly rich in phosphate dissolving microorganisms. Due to this, the phosphate assimilation by higher plants is an increased. The mycorrhizal fungus also increases the fertility of soil up to a moderate extent [42]. Many bacteria, fungi, and actinomycetes also release bound phosphorus in crop residues and soil organic matter which is ultimately available to plants. Also, mesophilic and hemophilic bacteria's actively participate in the mineralization of phosphorus [19] Warm temperature usually favors decomposition and due to this thermophilic species have an important role to play [26]. P^H of soil is also important in the process [12]**.** As like nitrogen, phosphorous is both mineralized and immobilized in the soil. Both these process operate in soil and are governed by the amount of phosphorus in the plant residues undergoing decomposition and the nutrients required for the associated microbial population. Phosphate is formed when the ratio narrows with time due to CO_2volatization (table 2).

Table 2: Some of the important PSM and their mode of action

PSM	**Metabolite forms (acids)**	**Reference**
PSB	NOT Determined(ND)	[35]
E. freundii	Lactic acid	[2]
A. niger, Penicillium sps	Lactic acid	[3]
Penicillium rugulosum	Citric, gluconic	[43]
Enterobacter intermedium	2-Keto gluconic	[44]
Aspergillus flavus, A. niger, penicillium canescens	Oxalic, Citric, gluconic, succinic	[45]
A. niger	Oxalic, Gluconic	[46]
P. fluorescens	Citric, malic, tartaric, gluconic	[47]
Arrhrobacter	Citric, malic, tartaric, gluconic	[48]
Enterobacter	Citric, alic,tartaric,gluconic,funaric	[48]
P. trivialis	Lactic, formic	[49]
Pseudomonas putida M5TSA, Enterobacter sakazakii, M2PFe and bacillus megaterium M1PCa		[50]
Enterobacter sps Fs 11	Malic,gluconic	[51]
A. nigerFS 1,Penicillium canescens FS23,Eupenicillium ludwigii FS 27, Penicillium islandicum FS 30	Citric,gluconic,oxalic	[52]
Psedomonas nitroreducens	Indole acetic acid	[53]

Table modified from: [4, 12]

Biotechnology and PSMs

Nowadays research has been focused on isolating different PSM and using them as biofertilizers as well. The role of biotechnology has a great impact on modifying these PSM by the use of genetic engineering. Researchers are now trying to characterize those PSM which are efficient in the phosphorous intake and promoting plant's growth as well [25] [12] concluded that PSM may be a sustainable approach for managing phosphorous deficiency in agricultural soils. Parmar *et al.*, 2014 reported the use of PSB to increase the fertility of sulfate soil which was later used for cultivating rice crop. Recently studies have demonstrated that 16 s RNA gene sequencing can give the phylogenetic relationship between PSB [54].

CONCLUSION

It is attractive to speculate that PSM through their mechanism of action can stimulate plant's nutritional intake capacity and growth as well. Because of phosphate solubilization, auxin, and HCN production ability, it is very obvious that these microorganisms (PSM) should be exploited more and could be used as an efficient alternative to inorganic phosphate fertilizers in future. Extensive research work needed to be done to achieve methods how to commercialize these PSM as biofertilizers. Greater attention should be paid towards the improvement of PSM strains.

CONFLICT OF INTERESTS

Declared none

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