

Topic: **PHOSPHATE SOLUBILIZERS**

Subject: Botany

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“Phosphorus (P) is one of the essential elements that are necessary for plant development and growth; it makes up about 0.2% of a plant’s dry weight. It is second only to nitrogen among mineral nutrients most commonly limiting the growth of crops (Azziz et al., 2012; Tak et al., 2012). On average, the phosphorus content of soil is about 0.05% (w/w); however, only 0.1% of this phosphorus is available for plant use (Zhu et al., 2011). Traditionally, the challenge of soil phosphorus deficiency is addressed by the application of phosphorus fertilizers. However, majority of the applied fertilizer phosphorus is not available to plants and the addition of inorganic fertilizers in excess of the amount that is commonly employed to overcome this effect can lead to environmental problems such as, groundwater contamination and waterway eutrophication (Kang et al., 2011). To counter this problem, quite a number of soil microorganisms capable of solubilizing/mineralizing insoluble soil phosphate to release soluble P and making it available to plants can be used. These microorganisms improve the growth and yield of a wide variety of crops. Thus, inoculating seeds/crops/soil with Phosphate Solubilizing Microorganisms (PSM) is a promising strategy to improve world food production without causing any environmental hazard.

These soil microorganisms enhance plant nutrient acquisition. They are involved in a wide range of biological processes including the transformation of insoluble soil nutrients (Babalola and Glick, 2012a). Some are capable of solubilizing and mineralizing insoluble soil phosphorus for the growth of plants. Apart from chemical fertilization, microbial P-solubilization and mineralization is the only possible way to increase plant available phosphorus. In the natural environment numerous microorganisms in the soil and rhizosphere are effective at releasing phosphorus from total soil phosphorus through solubilization and mineralization (Bhattacharyya and Jha, 2012). This group of microorganisms are referred to as Phosphorus Solubilizing Microorganisms (PSM). Many species of soil fungi and bacteria are able to solubilize phosphorus in vitro and some of them can mobilize phosphorus in plants (Zhu et al., 2011). PSM increases the bioavailability of soil insoluble phosphorus for plant use (Zhu et al., 2011). They solubilize insoluble inorganic (mineral) phosphorus and mineralize insoluble organic phosphorus (Sharma et al., 2013).” (Alori et al., 2017).

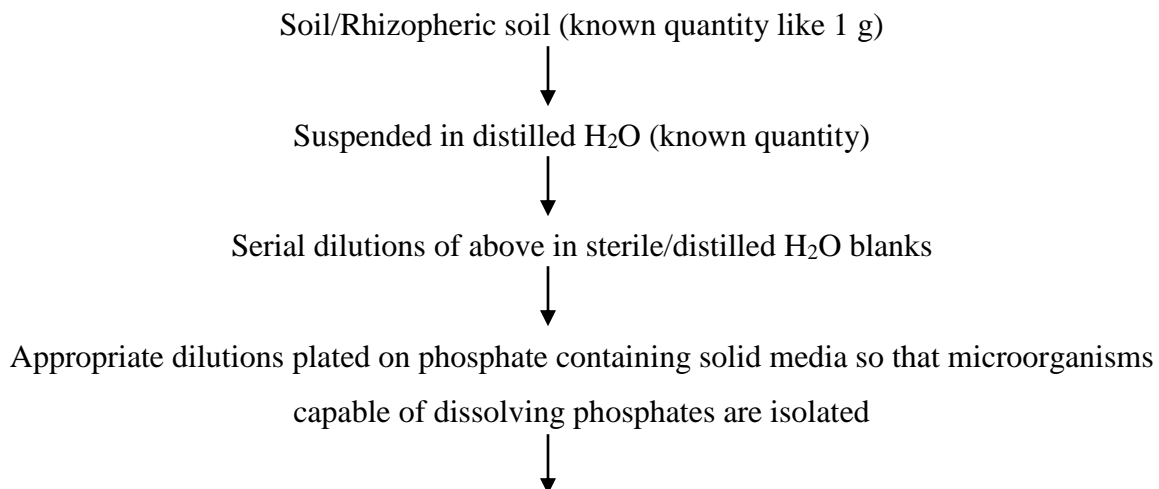
Phosphorus Solubilizing Microorganisms (PSM)/ Phosphate Solubilizers

“A large number of microbial organisms including bacteria, fungi, actinomycetes, and algae exhibit P solubilization and mineralization ability. Soil bacteria that have been reported to mobilize poorly available phosphorus via solubilization and mineralization include *Pseudomonas* spp., *Agrobacterium* spp., and *Bacillus circulans*. Other phosphorus solubilizing and mineralizing bacteria include various strains of *Azotobacter*, *Bacillus* etc.

The microbial fungi that function similarly include strains of *Achrothcium*, *Alternaria*, *Aspergillus*, *Cephalosporium*, *Fusarium*, *Glomus*, *Penicillium*, *Rhizopus*, *Saccharomyces*, etc. Soil fungi have been reported to be able to traverse long distances within the soil more easily than bacteria and may be more important to the solubilization of inorganic phosphate in soils as they typically produce and secrete more acids, such as gluconic, citric, lactic, 2-ketogluconic, oxalic, tartaric and acetic acid, than bacteria (Sharma et al., 2013). In addition, approximately 20% of actinomycetes could solubilize P, including those in the genera *Actinomyces*, *Micromonospora*, and *Streptomyces*. Algae such as cyanobacteria have also been reported to show P solubilization activity (Sharma et al., 2013).” (Alori et al., 2017).

Thus, PSM have the ability to bring insoluble P in soil to soluble forms by secreting organic acids which lower the pH and causes dissolution of bound forms of P. Few hydroxyl acids may chelate with Ca and Fe resulting in effective solubilization and utilization of phosphates. PSMs apply various approaches to make phosphorus accessible for plants to absorb. These include lowering soil pH, chelation, and mineralization (Kalayu, 2019).

Isolation and purification of PSM



Plates incubated (4 to 5 days)
↓
Transparent zone of clearing around microbial colonies indicates extent of P solubilization
↓
Such cultures are isolated, identified and extent of solubilization is determined quantitatively

Quantitative measurement of P solubilization in culture media

Now, cultures from above are selected and grown in 50 – 100 ml aliquots of Pikovskaya's¹ liquid medium for 6-17 days at 28⁰C (±2)

[If culture has fungi, it is filtered using Whatman no. 42 filter paper]

↓
Due to pigments, filtrate may be colored, for which 1 to 2 g of activated charcoal is added and shaken till filtrate becomes colorless

↓
Bacterial cultures are filtered through Whatman no. 1 filter paper so that insoluble phosphate is removed and then centrifuged at 10000 rpm for 10-15 mins.

↓
Filtration and centrifugation repeated until clear solution is obtained and made to volume (50-100 ml)

↓
From the above filtrate, 10 ml aliquot is taken and 2.5 ml of Barton's reagent² is added, vol. made to 50 ml

↓ After 10 mins
O.D./ Absorbance taken at 430 nm (Resultant color is read in colorimeter)

The phosphate content of culture is quantified by preparing a standard curve by dissolving KH₂PO₄, taking suitable aliquots and developing color by adding Barton's reagent (Koeing and Johnson, 1942).

¹ Has tricalcium phosphate as source of insoluble P + glucose+ yeast extract and other salts

² Barton's reagent

(A = 25gm ammonium molybdate to be dissolved in 400ml. H₂O.

B = 1.25 gm ammonium metavanadate in 300ml of boiling water, cooled and then 250 ml conc. HNO₃ added. Afterwards, A and B solutions are mixed and made up to a liter)

Mass multiplication and field applications of phosphate solubilizers

Selected bacteria grown in Pikovskaya's broth for 7 – 18 days at 28⁰C (±2) are mixed in suitable sterilized carrier (like peat soil, lignite powder etc)³



Mixture is cured for 7 days at 28⁰C (±2) in large trays covered with loosely fitting empty trays



Inoculant is then packed at rate of 300 g/ plastic packet and stored at 15 - 20⁰C until use (it is good to use it within a month to inoculate seeds).

These bio-fertilizers can be applied in fields mainly via seed treatment (most common), root dipping, and soil application.

³ Alternatively, “after the screening of the PSM bacterial strains like *Bacillus* spp and *Pseudomonas* spp from the pure culture slants, the bacterial strains were transferred to the liquid broth which are also the production media and as well as the starter culture for the growth of cells. Production media is that media in which the number of viable bacterial cells of that particular bacteria increases because that bacteria is grown in that particular media only. Thus in phosphate solubilising bacteria both the *Bacillus* and *Pseudomonas* spp. strains were grown in Pikovskaya's production media. Thus a 100ml of two separate conical flasks can be taken and PVK media is prepared after pH adjustments and autoclaved. Then inside the laminar airflow the pure cultures marked in the pure culture slants are transferred to the PVK production media conical flasks by the help of sterilized inoculating loop. Then the conical flasks were put in the rotary B.O.D shaker for 1 week. Then, for the mass production of PSM biofertilizer the inoculums from these starter cultures are transferred to larger flasks. The Phosphate solubilising bacterial strains in the starter cultures are thus needed to be grown in large scale for which their mass production is required. So larger conical flasks of 1000 ml are taken and then again starter cultures are transferred to these larger conical flasks containing the appropriate growth media in aseptic conditions for small scale production and for large scale production 1 litre of the starter cultures are put into the fermenter. Finally continuous agitation and proper aeration is done for about 1 week. The flasks is checked from time to time for the growth of the cell mass and that they remain free of any contamination. Then the conical flasks are stored in cool temperatures so that they can be mixed with proper carrier materials. Moreover, it is not advisable to keep the conical flasks for long time in storage because of the loss of cell load. The mass produced bacterial cell cultures of both *Bacillus* spp and *Pseudomonas* spp are taken out of storage and then the cell cultures are mixed with the sterilized carrier materials in individual beakers. The mixing of the carrier materials and the production media are in the ratio 2:1 where 1 part of production media is mixed with 2 parts of carrier material or in other words 30:60 ratio of both. It is done manually and under aseptic conditions. The biofertilizers are packed in polythene bags which are advised to be of 250 gm. Then the packets are left in room temperature for curing” (Roychowdhury et al., 2015).