

# INORGANIC P SOLUBILIZATION BY MICROORGANISMS AND THEIR POTENTIAL APPLICATIONS IN AGRICULTURE

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## ABSTRACT

Phosphorus (P) is essential for plant growth, but most of it in soil is insoluble and unavailable to plants. To increase crop yields, farmers use chemical fertilizers, causing soil pollution from the accumulation of insoluble P. Beneficial microorganisms called phosphorus-solubilizing microorganisms (PSMs) can change insoluble organic and inorganic phosphorus compounds into forms that plants can absorb. PSM offers an environmentally friendly and economically viable solution to address phosphorus deficiency and the limited P uptake in plants. Although PSM have been a research focus for decades, their application to increase soluble P in soil and improve crop productivity remains a topic of active study. This article seeks to deepen knowledge of how PSM can improve plant growth, decrease soil phosphorus contamination, and contribute to the future of sustainable agriculture.

## 1. INTRODUCTION

Sustainable agriculture is a solution to the food scarcity and hunger crises. According to the Food and Agriculture Organization (FAO, 2005), over 923 million people suffer from chronic hunger. Furthermore, this number is expected to rise to 9.3 billion by 2050 (Kishore et al., 2015). In light of this, a key requirement for an agricultural revolution is the utilization of underutilized or non-arable lands (low-yield lands) to expand crop areas. High agricultural yields depend on crop productivity and soil fertility. One of the main factors that reduces crop yields is nutrient deficiency. Various metabolic processes in plants at any growth stage can be affected by a lack of or unavailable nutrients in the soil. Plants with different genotypes vary in their ability to absorb nutrients from the soil by

converting non-assimilable substances into assimilable forms. In general, factors such as low solubility, poor mobility, or inherently low nutrient concentrations in different soil types cause nutrient deficiencies (Sessitsch et al., 2018).

Essential plant nutrients are relatively high, but their concentrations in the soil are insufficient to meet plant growth demands. Nutrients such as phosphorus (P), potassium (K), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) have limited solubility in soil. They are transported into the roots through processes such as diffusion, osmosis, active transport, or xylem transport. Nitrogen (N), phosphorus (P), and potassium (K) are among the key micronutrients necessary for plant development. Among these, nitrogen is abundantly available from the atmosphere.

Phosphorus is essential for the formation of molecules like DNA (deoxyribonucleic acid), RNA (ribonucleic acid), and phospholipids in both plant and animal cells. After nitrogen, phosphorus is the second limiting nutrient in agricultural production. Root surface area, crop yield, and disease resistance influence plants' uptake of nutrients, directly or indirectly. Phosphorus also plays a crucial role in photosynthesis, sugar breakdown, nutrient absorption, and transport. Plants deficient in phosphorus will experience slow growth (Sharma et al., 2013).

Moreover, soil only utilizes about 30% of chemical phosphate fertilizers while the remaining phosphorus is fixed as iron/aluminium phosphate in acidic soils or as calcium phosphate in neutral and alkaline soils (Lindsay et al., 1989). Studies have shown that the phosphorus accumulated in agricultural soils would be sufficient to maintain maximum crop yields worldwide for about 100 years if it were in a soluble form (Khan et al., 2009). Furthermore, only 1% of the total phosphorus in soil (400–4,000 P kg/ha in the top 30 cm) is absorbed by plants in each growing season (10–30 P kg/ha), indicating that the available phosphorus for plant uptake is very low (Blake et al., 2000). On the other hand, phosphorus is a finite resource, and based on current usage rates, it is estimated that global phosphorus reserves may be depleted in the next century (Cordell et al., 2009). Thus, the recognition of these underlying issues has led to the search for environmentally compatible and economically feasible alternative strategies to improve crop production in phosphorus-deficient soils (Zaidi et al., 2009). The use of phosphorus-solubilizing microorganisms (PSMs) in agricultural soils is considered an environmentally friendly alternative. Using PSMs as bio-fertilizers to enhance agricultural crop yields has become

an emerging research topic. This article aims to provide some insights into the existence of phosphorus in soil, the diversity of PSMs, phosphorus solubilization mechanisms, and their potential applications in sustainable agricultural production.

## 2. PHOSPHORUS-SOLUBILIZING MICROORGANISMS (PSMS)

Currently, scientists have discovered many microbial species capable of **phosphorus-solubilizing**, including bacteria, fungi, actinomycetes, and even algae. *Pseudomonas* and *Bacillus* are two common bacterial genera known for their phosphate-solubilizing abilities. Other bacterial genera capable of solubilizing phosphate include *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, *Delftia* sp. (Chen et al., 2003), *Azotobacter* (Kumar et al., 2001), *Xanthomonas* (De Freitas et al., 1997), *Enterobacter*, *Pantoea*, *Klebsiella*, *Vibrio proteolyticus*, and *Xanthobacter agilis* (Chung et al., 2005). Additionally, nitrogen-fixing *Rhizobia*, which converts atmospheric nitrogen into ammonia and transfers fixed nitrogen to the host plant, has also shown phosphate solubilizing activity (Zaidi et al., 2009). For example, *Rhizobium leguminosarum* bv. *Trifolii* (Abril et al., 2007), and *Rhizobium*, *Crotalaria* improve phosphorus nutrition in plants by mobilizing both inorganic and organic phosphorus. Researchers have isolated different phosphate-solubilizing bacteria (PSB) from nutrient-deficient environments, such as the halophilic bacterium *Kushneria sinocarni*, isolated from coastal sediments on the eastern coast of China, which is beneficial for agricultural soils affected by salt (Zhu et al., 2011).

In soil, phosphate-solubilizing fungi (PSF) account for about 0.1–0.5% of the total fungal population. Furthermore, PSF retains their phosphate-solubilizing activity even after researchers repeatedly subculture them under

laboratory conditions, unlike phosphate-solubilizing bacteria (Kucey, 1983). Generally, PSF produces more acids than bacteria and exhibits greater phosphate solubilizing activity (Venkateswarlu et al., 1984). Among the phosphate-solubilizing fungi, the genera *Aspergillus* and *Penicillium* are the most common (Khan & Khan, 2002), *Trichoderma* strains (Altomare et al., 1999) and *Rhizoctonia solani* (Jacobs et al., 2002) are also known to solubilize phosphate. Of the identified fungal species, many commonly found in agricultural soils, such as *Penicillium sp.*, *Mucor sp.*, and *Aspergillus sp.* have been shown to increase plant growth by 5-20% (Güneş et al., 2009). Vassilev et al. (2001) have also studied yeasts capable of phosphate solubilization, such as *Yarrowia lipolytica*, *Schizosaccharomyces pombe*, and *Pichia fermentans*.

The phosphate solubilizing ability of actinomycetes has attracted attention because this group of microorganisms not only survives in harsh environments, such as extreme heat but also has other potentials, such as producing antibiotics and phytohormones (Kucey, 1983), which can simultaneously benefit plant growth (Hamdali et al., 2008)). A study by Hamdali et al. (2008) showed that about 20% of actinomycetes can solubilize phosphate, including species from the common genera *Streptomyces* and *Micromonospora*. In addition to bacteria, fungi, and actinomycetes, algae, cyanobacteria, and mycorrhiza have also demonstrated phosphate-solubilizing activity (Widada et al., 2002).

### 3. MECHANISMS OF INORGANIC P SOLUBILIZATION BY MICROORGANISMS

#### 3.1. Producing organic acids

PSMs (phosphorus solubilizing microorganisms) can solubilize insoluble phosphate by secreting various organic acids. These organic acids lower the pH,

enhance the process of metal cation binding with phosphorus, form metal-phosphate ion complexes, and compete with phosphorus for adsorption sites. Organic acids are produced either through direct oxidation or mainly by oxidation and fermentation of organic carbon sources. These acids can directly solubilize phosphate or form complexes with Fe, Al, and Ca ions that are bound to phosphorus. Organic acids such as gluconic acid, oxalic acid, citric acid, lactic acid, tartaric acid, aspartic acid, etc., have been identified in studies using high-performance liquid chromatography (HPLC). Research results indicate that there is no direct correlation between the amount of acid produced and the amount of phosphorus released. The complex-forming ability of organic acids plays a crucial role in solubilizing insoluble inorganic phosphate (Sharma et al., 2013). In a study by Kucey (1983), it was demonstrated that the addition of 0.05 M EDTA to the culture medium of *Penicillium balaji* resulted in higher phosphate solubilization. Adding NaOH, on the other hand, inhibited the phosphate solubilizing activity of *Rhizobium* bacteria, which is related to 2-ketogluconic acid. This study emphasizes that the phosphate solubilization by microorganisms is mainly due to their ability to lower the pH of the environment.

#### 3.2 Reducing soil pH

Researchers have studied phosphate solubilization through cation acidification in various bacterial and fungal species. However, research on phosphate solubilization through alkalization remains limited. The secretion of organic acids by phosphate-solubilizing microorganisms (PSMs) is related to the release of protons ( $H^+$ ), which lowers the pH (Maliha et al., 2004). The release of protons or  $OH^-$  ions by PSMs can also affect soil pH. Acidification

and pH reduction are not the only mechanisms involved in phosphate solubilization, as lowering the pH does not always correlate with the amount of soluble phosphorus. High-performance liquid chromatography (HPLC) analysis of *Pseudomonas sp.* culture filtrate showed no formation of organic acids. Park et al. (2009) proposed that phosphate solubilization occurs through the secretion of ammonium ions ( $\text{NH}_4^+$ ) during nitrogen assimilation.

### 3.3. The release of protons ( $\text{NH}_4^+$ ) through assimilation and respiration

The amount of  $\text{NH}_4^+$  protons released affects soil pH, and microorganisms produce them through the utilization of nitrogen sources. Among various nitrogen sources such as asparagine, sodium nitrate, potassium nitrate, urea, and calcium nitrate, microorganisms that use  $\text{NH}_4^+$  as the sole nitrogen source exhibit higher phosphate solubilization compared to those using nitrate ( $\text{NO}_3^-$ ) (Sharan et al., 2008). The release of  $\text{NH}_4^+$  protons could be the sole mechanism for phosphate solubilization in some microorganisms. However, no studies have shown changes in pH or the amount of soluble phosphorus in other microorganisms. Park et al. (2009) suggested that other solubilization mechanisms exist. They also proposed that the release of  $\text{NH}_4^+$  protons depends on different mechanisms. Only a portion of assimilated  $\text{NH}_4^+$  is involved in phosphate solubilization.

### 3.4. Producing inorganic acids and $\text{H}_2\text{S}$

Inorganic acids such as HCl,  $\text{H}_2\text{SO}_4$ , and  $\text{HNO}_3$  are related to phosphate solubilization. Researchers have shown that *Enterobacter agglomerans* can solubilize phosphate in the form of hydroxyapatite via HCl mediation. Kim et al. (1997) studied *E. agglomerans* and genetically modified *E. coli*, and the results showed that adding acids such as HCl, citric acid, oxalic acid, and lactic acid to the culture

medium increased phosphate solubilization from hydroxyapatite, although citric acid was more effective in solubilizing phosphate than HCl. Strains of *Nitrosomonas* and *Thiobacillus* produce  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ , and solubilize phosphate. The release of inorganic phosphorus through  $\text{H}_2\text{S}$  is another mechanism. Some bacteria that produce  $\text{H}_2\text{S}$  react with iron phosphate, forming iron sulfate and releasing phosphorus. The sulfur oxidation process of these microorganisms increases the solubility of mineral phosphate by producing  $\text{H}_2\text{SO}_4$ , nitrate, and  $\text{CO}_2$ . However, these mechanisms are less accepted than the organic acid-mediated phosphate solubilization mechanism.

### 3.5. The indirect degradation pathway

Halvorson et al. (1990) suggested that rhizosphere microorganisms assimilate a large amount of phosphorus from the soil through absorption mechanisms. As a result, the balance between insoluble and soluble phosphorus is disrupted. Therefore, less soluble phosphate is lost indirectly. The soluble phosphorus inside PSMs cells is balanced with insoluble phosphorus, as the phosphorus content of microorganisms correlates with the breakdown of phosphorus-containing organic matter in the substrate. Although plants and other soil organisms will use some of the phosphorus released by PSMs degradation, environmental changes cause cells to die and release free phosphorus. Starvation, predation, drought, high humidity, freezing, or thawing can lead to a sudden increase in available phosphorus due to an unusually high rate of bacterial cell lysis (Butterly et al., 2009).

### 3.6. Direct degradation pathway

Goldstein (1995) proposed that phosphate solubilization primarily occurs through the extracellular oxidation process of PSMs in soil, with calcium phosphate providing a

significant amount of insoluble mineral phosphorus. Biochemical analyses on pH reduction and phosphate solubilization of *Burkholderia cepacia* DA 23 were conducted by Song et al. (2008).

### 3.7. Releasing exopolysaccharide (EPS)

Microorganisms mainly produce EPS in response to biofilm formation and biological stress. Microbial EPS are carbohydrate polymers secreted by certain bacteria and fungi on the outer surface of their cell walls. Previous studies have shown that EPS can form complexes with metals in soil (with the order of affinity  $Al^{3+} > Cu^{2+} > Zn^{2+} > Fe^{3+} > Mg^{2+} > K^+$  and play a role in phosphate solubilization in soil (Zaidi et al., 2009). Microbial EPS stimulates the release of tricalcium phosphate (TCP) through resonance with organic anions. Four PSB strains, such as *Enterobacter sp.* (EnHy-401), *Arthrobacter sp.* (ArHy-505), *Azotobacter sp.* (AzHy-510), and *Enterobacter sp.* (EnHy-402), were evaluated for their EPS role in solubilizing insoluble phosphate. The solubilization rate depends on the microbial source and EPS concentration (Yi et al., 2008).

### 3.8. Siderophore formation

Siderophores are iron-chelating compounds produced by most microorganisms in response to iron deficiency. Microorganisms produce these low-molecular-weight compounds (<10,000 Da) to combat stress under low iron conditions. Researchers have identified around 500 different siderophores, which plants and microorganisms utilize. Some PSMs also produce siderophores (Collavino et al., 2010).

## 4. POTENTIAL APPLICATIONS OF PSMs IN THE FUTURE

### 4.1. Use of PSM

Many studies have demonstrated the beneficial effects of PSMs when used alone. Lucy et al. (2004) found that stable phosphorus (P) uptake influences the growth and development of different crops. The success of microbial formulations depends mainly on their ability to colonize plant roots and survive competition with native microorganisms. Numerous studies have reported the positive impact of individual PSM species on plant growth. Phosphate solubilizing characteristics in bacteria such as *Rhizobium* and *Azotobacter* are examples of nitrogen-fixing microorganisms (Kumar et al., 2001). The effectiveness of phosphorus-solubilizing fungi (PSF) in enhancing inorganic phosphorus (Pi) availability for plant uptake and growth depends on soil type, especially the soil's ability to adsorb Pi. PSF studied individually or in combination with other fungi, can promote the development of crops such as mung bean, maize, wheat, faba bean, lentils, rice, and soybean. When combined with rock phosphate (RP) sources such as Mussoorie, Telesmi, etc., PSF is more effective in providing phosphorus and boosting growth, seed production, shoot height, seed weight, nitrogen and phosphorus accumulation, dry matter yield, root length, and root productivity.

### 4.2. Use of multiple phosphate solubilizing microorganisms (PSMs)

Both nitrogen (N) and phosphorus (P) are essential nutrients for plants. Combining nitrogen-fixing microorganisms with phosphate-solubilizing microorganisms (PSMs) can provide more significant benefits to plants than using each group alone. Nitrogen-fixing microorganisms like *Rhizobium* and PSF significantly impact crops such as wheat, mung beans, fava beans, peas, and others, improving seed yield, growth, and nutrient (N and P) uptake compared to control samples without the

combination of both microorganism groups. However, some studies suggest that this combination does not increase dry matter or total phosphorus (P) uptake (Kucey, 1987). Furthermore, high acidity conditions caused by PSF hinder the root colonization of *Rhizobium*, which explains the decrease in total nitrogen fixation.

#### 4.3. Combination of Arbuscular Mycorrhizal fungi (AMF) with PSMs

Arbuscular mycorrhizal fungi (AMF), the most common type of root fungi, form a symbiotic relationship with most major plant families. Soil microorganisms produce plant hormones that influence these fungi and affect AM formation (Jonas et al., 2004). AMF helps plants absorb nutrients, particularly phosphorus, from the soil more efficiently, improving stress resistance, reducing pathogen presence, and enhancing plant growth. The primary function of AMF is to improve phosphorus uptake by plants, as the fungal hyphae act as a bridge between the roots and soil microorganisms. This process facilitates the transfer of phosphate from the soil into the root zone. Phosphate produced by PSMs may not reach the root surface due to limited diffusion capacity. Research shows that AMF absorbs soluble phosphate, increasing the phosphorus supply to the plant. Specifically, adding AMF improves phosphate solubilization by both phosphate-solubilizing microorganisms and indigenous microorganisms (Medina et al., 2007). The simultaneous use of PSMs and AMF can overcome the limitations of PSMs in increasing plant-available phosphorus from the soil. During this interaction, the mycorrhizal fungi release large amounts of carbon into the root zone, which serves as a carbon source for PSMs. Reports show that crop yield and nitrogen concentration in wheat plants increased by over 50% and 90%, respectively, due to the synergistic effect of

*Glomus etunicatum* and *Burkholderia cepacia* BAM-6 (PSB) (Saxena et al., 2014).

#### 4.4. Using PSMs as biofertilizers

The application of PSMs can improve the effectiveness of phosphorus use in agricultural soils. The contributions of PSMs in solubilizing inorganic and mineral phosphates have been extensively studied (Aseri et al., 2009; Zaidi et al., 2009). Kalayu (2019) demonstrated that the phosphorus release rates from *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Tabriz Pseudomonas fluorescens* were 51%, 29%, and 62%, respectively. Similarly, *Glomus fasciculatum* and *Azotobacter* significantly improved the phosphorus (P), potassium (K), and nitrogen (N) uptake in mulberry plants compared to plants not supplemented with PSM (Baqual & Das, 2006). The application of *Pseudomonas* and *Bacillus* species enhanced phosphorus uptake and increased wheat seed yield (Walpola & Yoon, 2012). PSMs increase the availability of phosphorus without disturbing the biochemical composition of the soil. PSMs can be used on various crops, making it a potential source for effective biofertilizer formulations. Several studies report that the use of PSMs enhances growth, yield, and quality in a wide range of crops, including fruits, apples, maize, rice, mustard, oil palm, eggplant, chilli, soybean, wheat, sugar beet, sugarcane, mung bean, peanuts, beans, and potatoes (Pandey et al., 2006). Crops like cereals and legumes with adequate phosphorus supply show early germination and maturation of seeds (Sharma et al., 2013). PSMs also stimulate seedling root growth (Mehrvarz et al., 2008).

*Azotobacter* PSM improved sugarcane yield by 12.6% (Sundara et al., 2002) and wheat yield by up to 30% and 43% with *Bacillus* (Rodríguez & Fraga, 1999). Similarly, field trials using a combination of *Bacillus megaterium* and *Azotobacter*

*chroococcum* reported a 10-20% increase in wheat yield. Moreover, *Azospirillum* species increase yield in maize, millet, and wheat, while *Bacillus* species boost yield in peanuts, potatoes, millet, and wheat (Rodríguez & Fraga, 1999). Inoculating *Pseudomonas* strains significantly increased nodule count and dry weight compared to the control in peanut plants (Dey et al., 2004). Additionally, *Pseudomonas* showed beneficial effects on salt (NaCl) stress tolerance (Bano & Fatima, 2009). Yousefi et al. (2011) demonstrated that PSB, AMF, and their combination increased dry matter yield, seed count, and growth in wheat plants. The application of PSB and AMF resulted in the highest shoot dry weight and root dry weight, showing increased root growth, shoot length, and phosphorus uptake compared to the control. Furthermore, Afzal & Bano (2008) reported that supplementing both *Rhizobium* and PSB, without the need for phosphorus fertilizers, improved wheat seed yield by up to 20% compared to treatments with only phosphorus fertilizer. The impact of PSM on sugarcane yield and juice quality has also been studied, with phosphorus application becoming essential for sugarcane fertilization (Sundara et al., 2002). Many PSMs, such as *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis*, and *Pseudomonas striata*, have been proven effective as biofertilizers (Satyaprakash et al., 2017).

## 5. CONCLUSION

Researchers consider the addition of PSMs to soil an effective solution for converting insoluble phosphorus into a form accessible to plants. PSMs promote better plant growth and increase crop yields. *Bacillus*, *Pseudomonas*, *Rhizobium*, *Aspergillus*, *Penicillium*, and AMF are the most effective phosphorus-solubilizing microorganisms for enhancing phosphorus availability in soil. PSMs stimulate plant development by providing plant-available phosphorus and producing

plant growth hormones such as IAA and GA. Additionally, PSMs support plant growth by producing siderophores and enhancing nitrogen fixation. Furthermore, PSMs act as biological control agents against plant pathogens by producing antibiotics, hydrogen cyanide (HCN), and antifungal metabolites. Therefore, researchers consider PSMs a promising alternative to inorganic phosphorus fertilizers for supplying phosphorus to crops. The application of PSMs is an ecologically and economically viable approach. Thus, effective use of PSMs opens a new way to improve crop yields and maintain soil fertility. However, understanding the phosphorus solubilizing mechanisms of soil microbial communities determines the sustainability and practical application of PSMs. Therefore, extensive and consistent research efforts are needed to isolate and identify more PSM species with higher efficacy under field conditions to achieve sustainable and environmentally friendly agriculture.

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## VI SINH VẬT HÒA TAN LÂN VÔ CƠ VÀ TIỀM NĂNG ỨNG DỤNG TRONG NÔNG NGHIỆP

### TÓM TẮT

*Phosphor (P) là một trong các chất dinh dưỡng đa lượng cần thiết cho hoạt động sinh trưởng và phát triển của thực vật. Trong đất P tồn tại dưới hai dạng hữu cơ và vô cơ, hầu hết chúng thường khó tan, do đó thực vật không hấp thu được. Bên cạnh đó, để đáp ứng nhu cầu lương thực ngày càng tăng, việc sử dụng phân bón hóa học quá mức để tăng năng suất cây trồng, dẫn đến làm ô nhiễm môi trường đất do tích tụ khá nhiều lân khó tan. Vi sinh vật hòa tan phosphate (PSM) là một nhóm các vi sinh vật có lợi, có khả năng thủy phân các hợp chất phosphor không hòa tan dạng hữu cơ và vô cơ thành dạng P hòa tan mà thực vật có thể hấp thu. PSM là giải pháp thân thiện với môi trường và hợp lý về mặt kinh tế để khắc phục sự thiếu hụt và sự khó hấp thụ P của thực vật. Mặc dù PSM đã là một chủ đề nghiên cứu trong nhiều thập kỷ, nhưng việc ứng dụng PSM để tăng P dễ tan trong đất và cải thiện năng suất cây trồng là vấn đề đang được quan tâm nghiên cứu. Mục đích của bài viết này là để mở rộng sự hiểu biết về vai trò của PSM trong việc cải thiện sự phát triển của thực vật, giảm ô nhiễm P trong đất và tiềm năng ứng dụng PSM để hướng đến một nền nông nghiệp bền vững trong tương lai.*

**Từ khóa:** Lân hữu cơ, lân vô cơ, nông nghiệp bền vững, phosphor, vi sinh vật