

FACTORS AFFECTING PHOSPHORUS SOLUBILIZATION AND THE ROLE OF PHOSPHATE-SOLUBILIZING BACTERIA IN AGRICULTURE

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ABSTRACT

The study involved gathering relevant literature on inorganic phosphorus-solubilizing bacteria and their role in agriculture. The review provides basic knowledge about the factors influencing the phosphorus solubilization process by bacteria in the soil, which in turn can lead to applications of these bacterial groups. Phosphorus (P) is crucial in regulating plant metabolism and health. Phosphorus exists in the soil in an insoluble form, and plants cannot utilize insoluble phosphate forms in the soil. The application of phosphate-solubilizing bacteria (PSB) is an effective method for converting insoluble phosphorus into soluble forms. However, the phosphorus solubilization efficiency of PSB is significantly affected by environmental factors such as temperature, pH, salt concentration, substrate concentration, type of microorganisms, incubation time, etc. Therefore, it is necessary to control the factors that affect the phosphorus solubilization process of PSB in the soil, creating optimal conditions for the activity of the PSB group during their application.

1. INTRODUCTION

Nitrogen (N), phosphorus (P), and potassium (K) are three essential nutrients required for plant growth. Among these, phosphorus is considered the second most limiting nutrient after nitrogen. Despite various forms of P in the soil, its availability to plants remains limited. Phosphorus deficiency is often compensated by the application of chemical fertilizers. However, chemical fertilizers are expensive and not environmentally friendly. The improper use of chemical fertilizers over extended periods leads to a reduction in the activity of soil microorganisms and causes an imbalance in the microbial ecosystem [1]. Currently, utilizing microorganisms to improve phosphorus absorption is considered the most effective solution. Phosphate-solubilizing

microorganisms (PSM) help provide soluble phosphorus, promote plant growth, perform biological control within plants and aid in the protection of crops against pathogens.

The process of phosphorus fixation is the conversion of phosphate from an insoluble form to a soluble form [2]. Phosphorus fixation occurs through two main mechanisms: First, phosphate is adsorbed onto the mineral surfaces of the soil. Second, phosphate is precipitated by binding to Al^{3+} and Fe^{3+} ions in the soil. In tropical countries, soils with a relatively high level of fixed phosphorus cover approximately 1.018 million hectares [3]. This is one reason why a significant amount of soluble phosphorus is added to most agricultural soils through the use of chemical fertilizers. The result is high production costs. This leads to negative

environmental impacts, including the degradation of terrestrial and aquatic resources, as well as freshwater and marine ecosystems. Additionally, increased phosphorus concentrations have been identified as a primary factor contributing to the eutrophication of surface waters, potentially leading to harmful algal blooms [4]. The use of chemical fertilizers will affect the microbial diversity in the soil, leading to a decrease in crop yields due to the loss of a significant amount of beneficial microorganisms. Phosphorus deficiency in the soil affects microbial respiration processes. For example, the lack of streptomycin sulfate affects fungal activity and bacterial population growth in soil. The application of triple superphosphate at 94 kg/ha significantly impacts microbial respiration and CO₂ metabolism [5].

Microorganisms are an integral part of the phosphorus cycle in soil and are crucial for converting phosphorus from insoluble to soluble forms. PSM can convert inorganic and organic phosphorus in the soil for plant absorption through solubilization and mineralization mechanisms [1]. It is important to identify the actual phosphorus solubilization mechanisms of PSM to optimize their usage under different environmental conditions. Therefore, a deeper understanding of the factors affecting phosphorus solubilization is essential. This knowledge can help propose effective and efficient strategies for utilizing PSM, leading to the development of beneficial microbial formulations for agricultural use.

2. INORGANIC PSB

Bacteria are known for their ability to release phosphorus bonds from various sources (PSB) present in moist soils, and they can make up to 40% of the microbial population in agricultural soils. Aerobic and anaerobic phosphorus-solubilizing bacteria are commonly present in large quantities in the soil, plant root zones, and even marine

environments [6]. Common bacterial groups involved in phosphate solubilization include *Bacillus*, *Pseudomonas*, *Azotobacter*, *Burkholderia*, and *Rhizobium* [7]. Approximately 60% of *Bradyrhizobium* strains are capable of solubilizing phosphorus [8]. Some *Rhizobia* strains capable of phosphate solubilization can colonize roots like other plant growth-promoting rhizobacteria (PGPR) and enhance the productivity of both leguminous and non-leguminous plants.

Researchers have used *Azotobacter chroococcum* to enhance wheat plant growth by solubilizing inorganic phosphate from tricalcium phosphate (TCP) and Mussoorie rock phosphate (MRP). The study showed that 18% of *Pseudomonas fluorescens* species could solubilize TCP by forming dissolution zones on Pikovskaya agar. *Pseudomonas fluorescens* enhances root surface, and phosphate solubilization, improves nutrient uptake and increasing seedling biomass. *Pseudomonas fluorescens* and *Rhizobia* solubilize organic and inorganic phosphates [9]. Strains such as *P. fluorescens* NJ-101, *P. fluorescens* EM85, *P. aeruginosa*, *Pseudomonas* sp., *P. chlororaphis*, *P. savastanoi*, *P. pickettii*, *P. lulea* OK2, *P. rhizosphaerae* LMG 1640, *P. graminis* DSM 11363, *P. striata*, and *P. corrugata* are effective at phosphate solubilization. Active bacteria capable of phosphate solubilization that colonize mycorrhizal hyphae may indirectly contribute to phosphorus uptake by mycorrhizae. Studies also show that these bacteria inhabit the mucus of hyphae, the hyphal plane, the hyphal cell walls, and even the hyphae and spores. In addition to solubilizing phosphorus, these bacteria may perform other functions simultaneously [10].

3. FACTORS AFFECTING INORGANIC PHOSPHORUS SOLUBILIZATION

3.1. Concentration of H⁺ ions (pH)

The pH of the medium in which PSM grow is one of the factors that influence their growth

and metabolic activity. Functionally, the optimal pH for maximum inorganic phosphorus solubilization by bacteria is neutral or slightly acidic. This is generally true because, in most cases, acidification is considered the primary method of phosphorus solubilization by bacteria [11]. Therefore, the high phosphorus solubilization capacity of bacteria is often associated with a reduction in the pH of the environment. At the same time, there is a significant negative correlation between pH and inorganic phosphorus solubility, as observed in the study by Wani. Moreover, studies have shown a significant correlation between the pH measured after culturing, the titratable acid content, and the amount of soluble phosphorus when 42 bacterial strains were cultured in media containing rock phosphate (RP) and Ca-P. Further research has demonstrated that phosphorus-solubilizing fungi and PSB cultured in liquid Pikovskaya (PVK) medium with low pH exhibited high phosphorus solubilization efficiency, and phosphorus solubilization increased as the pH value decreased. Interestingly, both bacteria (*Pseudomonas aeruginosa* and *Bacillus subtilis*) and fungi (*Aspergillus niger*, *Penicillium* sp., and *Micrococcus* sp.) showed maximum phosphorus solubilization at pH 3.0 at 28°C and 37°C [12].

3.2. Temperature

The temperature has significant effects on the phosphorus solubilization ability of heat-tolerant PSB found in nature, such as *Bacillus subtilis*, *Acidithiobacillus caldus*, *Bacillus smithii*, and *Pseudomonas fragi* [13]. At low temperatures, microbial activity decreases, but as the temperature rises to the optimal level, microorganisms adjust their biological processes accordingly. Generally, researchers can only culture thermophilic PSB in thermophilic environments. Some heat-tolerant PSB and cold-adapted PSM also show phosphate solubilizing activity. *Bacillus subtilis* and *Bacillus circulans* demonstrated stable phosphorus solubilization at 45°C, likely because their enzyme systems are heat-resistant [14].

In tropical countries, soil temperatures during summer can reach up to 50°C, which highlights the need for formulations that can remain stable at higher temperatures in the soil. Therefore, isolating PSM that can tolerate high temperatures is essential. To meet this requirement, Khan et al. (2010) tested various PSM for their ability to solubilize phosphorus at temperatures of 35°C, 40°C, and 45°C. Although there were significant variations in phosphate solubilizing activity, the highest phosphorus solubilization occurred at 45°C. Heat-tolerant bacterial strains that demonstrated phosphate solubilization at high temperatures include *Bacillus subtilis* and *Bacillus circulans* [15]. Panda et al. (2013) found that *Pseudomonas fluorescens* solubilized phosphorus at 35°C, while *Pseudomonas corrugata*, isolated from the temperate region of Sikkim (Himalayas), solubilized TCP at both low and moderate temperatures. The maximum phosphate solubilization occurred at 21°C but it could also solubilize TCP at 28°C [16]. The phosphate solubilization ability of *Pseudomonas lurida* M2RH3 was assessed at three different incubation temperatures, showing a steady increase in the concentration of soluble phosphorus as the pH decreased in the culture medium [17]. *Pantoea dispersa*, a cold-adapted species, and *Exiguobacter acetylicum* effectively solubilized phosphorus at lower temperatures. The strain *Serratia marcescens* SRM (MTCC 8708) was able to solubilize about 28 mg P/mL in the medium (NBRIP) at 4°C. Gulati et al. (2008) also isolated different *Pseudomonas* strains from cold regions capable of solubilizing TCP, Mussoorie rock phosphate (MRP), Udaipur rock phosphate (URP), and North Carolina rock phosphate (NCRP). Using PSM increases the rate of decomposition of agricultural and animal waste, which enhances phosphorus uptake efficiency. Heat-tolerant PSB releases more soluble phosphorus compared to actinomycetes or fungi [18].

3.3. Carbon sources and energy sources

Various carbon sources, such as glucose, galactose, fructose, starch, and mannitol, influence the growth, development, and

phosphate-solubilizing activity of PSM. These carbon sources are found in the root zone. Many plants synthesize them through photosynthesis [19]. These compounds serve as carbon and energy sources for many soil bacteria, including PSM [11]. Song et al. (2008) further demonstrated the impact of carbon sources on phosphate solubilization activity, by using different sugars such as glucose, sucrose, or maltose to assess the phosphate solubilization activity of *Burkholderia cepacia* (DA23). Among all the sugars tested, sucrose resulted in a two-fold lower phosphate solubilization compared to glucose in a medium containing calcium phosphate [16]. Mechanistically, the phosphate solubilizing efficiency of microorganisms is related to their ability to release organic acids. However, the nature and quantity of these acids vary between different sugars and bacteria. Phosphate solubilization activity increases with higher concentrations of sugars in the culture medium. Increasing glucose concentrations from 1% to 3% significantly enhanced the RP solubilization of PSM, as higher glucose levels lead to increased acid production, a key factor in phosphate solubilization [20]. In other studies, further increasing glucose concentrations from 1% to 5% (w/v), significantly enhanced phosphate solubilization, with the most significant increase observed at 3% glucose, while a decline occurred at 5% glucose [21].

3.4. Nitrogen source

Nitrogen like many other nutrients, affects the growth and function of PSM. Nitrogen is involved in various metabolic processes, such as the synthesis of amino acids, proteins, and nucleotides. Microorganisms utilize nitrogen in forms such as ammonium, nitrite, nitrate, or amino, depending on the enzymes present in the cells, which significantly influences phosphate solubilization activity. Researchers recently conducted an in vitro experiment to assess the effect of different nitrogen forms (NH_4^+ or NO_3^-) on the RP solubilization of *Mortierella* sp. The study showed that when NH_4Cl or NH_4NO_3 were

present in the culture medium, the pH decreased significantly from an initial value of 7.6 to 3.4 and 3.7, while KNO_3 only reduced the pH to 6.7. The pH reduction caused the solution to solubilize more phosphorus [22].

3.5. CaCO_3 and aeration

Farmers often adjust the pH of acidic soils, they use pyrite and gypsum to improve alkaline or saline soils. However, adding CaCO_3 to the medium significantly reduces the phosphorus solubilization capacity of bacteria and fungi. For example, *Enterobacter intermedium*, isolated from grass root zones, has strong phosphate solubilizing ability, and soluble phosphorus concentration decreased significantly to 200-250 mg/L in a medium treated with 1% CaCO_3 , compared to 1,000 mg/L in the medium without CaCO_3 . Moreover, bacteria oxidize glucose to gluconic acid, and subsequently to 2-keto-gluconic acid (2-KGA). Similarly, the addition of CaCl_2 , CaCO_3 , and $\text{Ca}(\text{OH})_2$ to the medium reduced the phosphorus solubilization of *Rhizobium* and *Bradyrhizobium* from RP, as CaCO_3 increased the pH of the medium, inhibiting bacterial growth, leading to less phosphorus solubilization [23].

Aeration is another important factor that contributes to phosphorus solubilization by bacteria. The *Pseudomonas striata* and *Aspergillus awamori* strains showed improved phosphorus solubilization in shaken cultures compared to static cultures. The increased phosphorus solubilization in aerated cultures was due to enhanced oxygen supply during cultivation. Under aeration conditions, the phosphorus concentration increased from 349 ppm to 1,675 ppm, while in non-aerated conditions, it rose from 242 ppm to 1,164 ppm [24].

3.6. Microbial species and incubation time

Microbial composition also influences the degree of P solubilization, as microbial communities are unevenly distributed and display varying P solubilizing abilities. Among microbial communities, the genera *Bacillus* (e.g., *B. polymyxa*) and *Pseudomonas* (e.g., *P. striata*) display the highest P solubilizing activity,

followed by *Penicillium* and *Aspergillus*, *Streptomyces* exhibits the lowest P solubilizing activity. Generally, fungi are more effective at dissolving phosphorus in liquid media than bacteria, actinomycetes, and yeasts [25]. This is because fungal hyphae can attach to P mineral particles and move more easily over greater distances in the soil than bacteria, thus playing a significant role in P solubilization in soil. Pigment-producing and non-pigment-producing bacterial strains show differences in P solubilization ability [26]. Many P-solubilizing bacteria lose their ability to solubilize P after frequent subculturing [25].

Incubation time plays a crucial role in the production of organic acids. This, in turn, affects the P solubilization process. P solubilization ability in the culture medium increases progressively with incubation. A decrease in P solubilization may occur after an incubation due to nutrient depletion, the production of toxic metabolites in the growth medium, or cell autolysis [11].

3.7. Substrate type

Phosphate-solubilizing microorganisms can solubilize various forms of phosphorus from compounds such as DCP, TCP, Fe and Al phosphates, bone meal, apatite, and different RP. In one study, *Burkholderia cepacia* DA23 showed higher phosphorus solubilization with TCP and hydroxylapatite than with Al-P, and its solubilization increased as the amount of insoluble phosphorus increased. The P solubilizing ability of bacteria depends on three factors: the mineral composition of apatite, the presence of impurities, and particle size. Substituting Mg or Na for calcium salts enhances the solubilization of RP. The solubilization efficiency decreases as the RP particle size becomes finer rather than coarser. The P solubilization efficiency of *A. awamori* and *P. striata* reaches its maximum when the RP particle size ranges between 30-59 and 60-99 microns. However, complete solubilization of RP never occurs because a portion of RP is bound so strongly that concentrated HCl or H₂SO₄ cannot dissolve it [20].

3.8. Salt concentration

There are various types of salts in the soil that can hinder water absorption and simultaneously be toxic to soil microbial communities. Phosphate-solubilizing bacteria thrive in high-salinity environments such as seawater [27]. When grown in media with varying concentrations of salt (NaCl), *Pseudomonas aeruginosa*, *P. putida*, *P. cepacia*, and *P. fluorescens* exhibited optimal P solubilization at 0–1.25% NaCl, higher NaCl concentrations delayed the P solubilization process [28]. In one study, the effect of salt concentrations (0%, 2%, 4%, 6%, and 8%) on the P solubilization ability of *Bacillus* strains isolated from wheat root zones of three wheat cultivars from arid and semi-arid regions in Algeria was investigated [29]. The researchers cultured the bacteria in NBRIP medium with varying salt concentrations and observed a gradual decrease in solubilizing activity as the salt concentration increased. However, the response of *Bacillus* to salt depended on the bacterial strain, with strains D1 (121.84 µg/mL) and D13 (112.83 µg/mL) demonstrating maximum P solubilization compared to other strains. Interestingly, strains B8 (58.8 µg/mL), BA5 (78.7 µg/mL), and BA11 (88.5 µg/mL) solubilized more P at 2% NaCl and exhibited high solubilization while reducing the pH of the medium with higher NaCl concentrations. Some strains, such as B14 (31.94 µg/mL), B18 (78.54 µg/mL), BA7 (75.80 µg/mL), and BA12 (35.26 µg/mL), showed similar results even at 4% NaCl. Banerjee et al. (2010) also showed optimal P solubilization at a salt concentration of 2.5%. The reduced P solubilization and microbial growth in high-salt media can be explained by the following factors: (i) Salts negatively impact growth and cell proliferation, which results in a loss of effective solubilization ability. (ii) Chloride ions (Cl⁻) isolate or neutralize protons or acids produced in the medium, thereby decreasing P solubilization activity [30]. The decrease in P solubilizing activity of PSM with increasing NaCl concentration may occur because the

microorganisms are exposed to the hyperosmotic conditions of salt. This exposure reduces the water activity within their cytoplasm. Soluble substances (NaCl) increase the osmotic pressure of the medium, causing intracellular dehydration while increasing the intracellular osmolarity [31]. According to Dey et al. (2021), *Kushneria sp. YCWA18* isolated from the sediment of Daqiao saltern in China, can grow on a solid medium containing 20% (w/v) NaCl [3]. Yue et al. (2019) found that *Bacillus altitudinis* WR10, isolated from wheat (*Triticum aestivum* L.) roots, can tolerate a salt concentration of 12% NaCl [32]. The study of Abdelmoteleb and Gonzalez-Mendoza (2020) showed that two strains, *B. megaterium* AL-18 and *B. cereus* AL-19, isolated from the root soil of *Tamarix ramosissima*, can survive and solubilize inorganic phosphate up to 14% NaCl. The maximum solubility of tri-calcium phosphate (TCP) for both strains was observed at 6% NaCl and pH 7 [33].

4. ROLE OF PSM IN SOIL

In addition to their ability to solubilize P, PSM also provide several beneficial effects on various types of soil. PSM help stabilize soil structure and enzyme activity, and support the activity of soil microbial communities [34].

4.1. PSM help stabilize soil

Microorganisms influence soil properties both mechanically and by secreting polysaccharides into the environment. Bearden and Petersen (2000) proposed that PSM enhance soil structure through the production of polysaccharides. PSM have been shown to increase soil nutrient synthesis and stabilize water-soluble carbon and carbohydrates in the root zone [35].

4.2. PSM enhance soil enzyme activity

PSM increase the enzyme content and activity of enzymes in the soil. PSMs improve the activity of enzymes such as dehydrogenase, phosphatase, and β -glucosidase, as well as antioxidant enzymes like ascorbate peroxidase (APOX), glutathione reductase (GR), superoxide

dismutase (SOD), and catalase (CAT) in plants grown in contaminated soils [36]. Furthermore, the simultaneous interaction between *Arbuscular Mycorrhizal* fungi (AM) and PSM have improved enzyme activity in soils with different salinity levels [37]. The increase in enzyme activity in both soil and plants enhances nutrient content and re-mineralizes nutrients for the soil.

4.3. PSM improve soil bacterial communities

Arbuscular Mycorrhizal (AM) fungi affect the microbial community both directly and indirectly. The addition of PSM has increased the fatty acid content in all microbial groups. This increase is due to the provision of an increased carbon source. The microbial community remains unaffected in the presence of only AM fungi. The growth and activity of AM fungi were found to increase in agricultural waste treated with P-solubilizing *A. niger*. Another study demonstrated that beetroot treated with *A. niger* increased the bacterial population in the root system [36]. The application of PSM seems essential for enhancing microbial communities, soil structure, and soil properties.

4.4. PSM enhance soil restoration

Treating areas contaminated with heavy metals by adding soluble phosphates or insoluble phosphate sources, such as RP, is an interesting approach. Hydroxyapatite is an effective metal-fixing agent. This method stabilizes heavy metals using hydroxyapatite, synthetic compounds, natural apatite, and rock phosphate. However, the heavy metals in the soil are influenced by organic matter [38]. Different microorganisms can mobilize metals through autotrophy, heterotrophy, leaching, iron excretion, and methylation. Acidification through organic acids and siderophores can provide protons and anions that form metal complexes, leading to the release of metals. Medina et al. (2006) demonstrated that sugar beets treated with *A. niger* improved growth and nutrition in soil contaminated with heavy metals (Zn and Cd). The mineralization by P-solubilizing microorganisms and the *G. reueae* fungus accelerated the growth rate of

sugar beet plants by 28 times compared to untreated plants [39].

4.5. Effects of PSM on plant growth in greenhouse and field conditions

Solutions for plant growth need to be improved to reduce the burden on the environment and other resources. PSM enhance plant growth in agriculture, forest restoration, and environmental management. At the current extraction rate of 7,100 million tons per year, phosphate reserves may be depleted in about 500–600 years. In India, 98% of arable land lacks available P, necessitating the annual importation of two million tons of rock phosphate. PSM help increase crop yields; however, variability and inconsistencies across laboratory, greenhouse, and field studies limit the results. Soil is a variable environment, making it difficult to achieve the expected results at times. PSM can increase crop yields by up to 70% while reducing chemical fertilizer use by 25%. Several studies show that beneficial formulations in the rhizosphere significantly enhance plant growth rates. The combination of multiple PSM is more effective for plant growth under varying conditions of agricultural ecosystems. In agroforestry, combining two or more microbial species leads to better plant growth than using just one species [40], [41].

5. CONCLUSION

The optimal environmental conditions are critical factors that influence the phosphorus solubilization process of PSB strains. Key factors such as temperature, pH, salinity, substrate concentration, C and N sources, CaCO₃ levels, and oxygen concentration determine the ability of microorganisms to solubilize phosphorus. A thorough understanding of how environmental factors affect the structure and function of PSM is essential for developing and modelling fertilizers for transfer to end-users. To obtain PSM with high phosphorus solubilization activity, it is crucial to analyze samples from diverse sources under challenging environmental conditions to identify the most suitable strains. This approach can help improve plant nutrition

under stress conditions and potentially increase crop yields, contributing to sustainable agricultural production.

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CÁC YẾU TỐ ẢNH HƯỞNG ĐẾN QUÁ TRÌNH HÒA TAN PHOSPHATE VÀ VAI TRÒ CỦA VI KHUẨN PHÂN GIẢI PHOSPHATE TRONG NÔNG NGHIỆP

ABSTRACT

Nghiên cứu này bao gồm việc thu thập tài liệu liên quan đến vi khuẩn phân giải phospho vô cơ và vai trò của chúng trong nông nghiệp. Bài tổng quan cung cấp kiến thức cơ bản về các yếu tố ảnh hưởng đến quá trình phân giải phospho của vi khuẩn hòa tan lân trong đất, từ đó ứng dụng nhóm vi khuẩn này. Phospho (P) rất quan trọng trong việc điều chỉnh quá trình trao đổi chất và dinh dưỡng của cây trồng. Phospho tồn tại trong đất dưới dạng muối phosphate không tan và cây trồng không thể sử dụng các dạng phosphate không tan trong đất. Việc sử dụng vi khuẩn phân giải phosphate (PSB) là một phương pháp hiệu quả để chuyển đổi phosphate không tan thành các dạng dễ tan. Tuy nhiên, hiệu quả phân giải phosphate của PSB bị ảnh hưởng đáng kể bởi các yếu tố môi trường như nhiệt độ, pH, nồng độ muối, nồng độ chất nền, loại vi sinh vật, thời gian ủ... Do đó, cần phải kiểm soát các yếu tố ảnh hưởng đến quá trình phân giải phosphate của PSB trong đất, tạo ra các điều kiện tối ưu cho hoạt động của nhóm vi khuẩn PSB trong quá trình ứng dụng.

Từ khóa: Chế phẩm vi sinh, quá trình hòa tan phospho, trao đổi chất của cây trồng, vi khuẩn phân giải phospho (PSB), yếu tố môi trường