Int.J.Curr.Res.Aca.Rev.2018; 6(11): 48-55

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) Volume 6 Number 11 (November-2018)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2018.611.006

Review on Role of Phosphate Solubilizing Microorganisms in Sustainable Agriculture

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Abstract

Most of soil microorganisms are known for their ability to solubilize insoluble phosphorous compounds for releasing soluble phosphate to make phosphorous available for plant absorption. Releasement of this soluble phosphorous is very important in increasing plant growth and plant product yields. Inoculating plant seeds with phosphate solubilizing microorganisms is very effective and environmentally useful to decrease the side effect of chemical fertilizers on soil microbes and other organisms. A large number of microbial organisms including bacteria, fungi, actinomycetes, and algae exhibit Phosphate solubilization and mineralization ability. Many soil bacteria have been recognized for their ability to solubilize insoluble phosphate. These strains of bacteria include: *Pseudomonas spp.*, *Agrobacterium spp.*, and *Bacillus circulans*. Other phosphorus solubilizing and mineralizing bacteria include various strains of *Azotobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Kushneria, Paenibacillus, Ralstonia, Rhizobium, Rhodococcus, Serratia, Bradyrhizobium, Salmonella, Sinomonas* and *Thiobacillus*. The strains of fungi with P solubilization ability are: *Achrothcium, Alternaria, Arthrobotrys, Aspergillus, Cephalosporium, Cladosporium, Curvularia, Cunninghamella, Chaetomium, Fusarium, Glomus, Helminthosporium, Micromonospora, Mortierella, Myrothecium, Oidiodendron, Paecilomyces, Penicillium, Phoma, Pichia fermentans, Populospora, Pythium, Rhizoctonia, Rhizopus, Saccharomyces, Schizosaccharomyces, Schwanniomyces, Sclerotium, Torula, Trichoderma, and Yarrowia.* Different species of fungi can solubilize rock phosphate, aluminum phosphate and tricalcium phosphate. For application of phosphate solubilizing Microorganisms to seeds, the seeds should be surface sterilized, rinsed with sterile water and dried. The surface disinfected seeds can be coated by soaking seeds in liquid culture medium of phosphate solubilizing fungi for 2 hr. While inoculation of the seed using plant and both *Aspergillus niger + Penicillium notatum* is recommended. Therefore, the application of phosphate solubilizing fungi is recommended as a sustainable way for increasing crop yield.

Article Info

Accepted: 18 October 2018
Available Online: 20 November 2018

Keywords

Phosphate solubilizing
Microorganisms
Sustainable agriculture.

Introduction

Phosphate solubilizing microorganisms (PSMs) are organisms that offer an ecologically acceptable mean for converting insoluble phosphate to soluble forms making them available for plants to absorb(1). Phosphate solubilizing microorganisms can improve the growth and yield of a wide variety of crops. Inoculating seeds/crops/soil with Phosphate Solubilizing Microorganisms (PSM) is a promising strategy to improve world food production without causing any environmental hazard (2). Phosphorous is very important nutrient in plant growth and crop yield. Although abundant in soils, in both organic and inorganic forms, its availability is restricted as it occurs mostly in insoluble forms (3). The production of chemical phosphatic fertilizers is a highly energy-intensive process requiring energy worth US $ 4 billion per annum in order to meet the global need. The situation is further compounded by the fact that almost 75–90% of added phosphatic fertilizer is precipitated by metal cation complexes present in the soils. According to Khan *et al.*, it has been suggested that the accumulated phosphates in agricultural soils are sufficient to sustain maximum crop yields worldwide for about 100 years (4). Using phosphate solubilizing microorganisms to increase the yield of the crop is very advanced and important naturally available mechanism. Because, using PSM will not affect the soil in any direction(5). The organisms possessing a phosphate-solubilizing ability can also convert the insoluble phosphatic compounds into soluble forms in soil and make them available to the crops (4). The majority of crop plants have been found to be positively affected by the association with rhizospheric microorganisms under phosphorus-deficient conditions. This association could result either in improved uptake of the available phosphates or rendering unavailable phosphorus sources accessible to the plant. Arbuscular mycorrhizae (AM) belong to the former category, while the latter category includes numerous bacteria and fungi capable of solubilizing insoluble mineral phosphate (4). The objective of this review is to address the importance of phosphate solubilizing microorganisms, how to inoculate these PSM to seeds and mechanisms used by those microorganisms to solubilize insoluble phosphate.

Phosphate Solubilizing Microorganism (PSM)

Many species of microorganisms including bacterial species, fungal species, actinomycetes, and algae exhibit phosphate solubilization and mineralization ability. Soil bacteria with ability of phosphate solubilization and mineralization include *Pseudomonas spp., Agrobacterium spp., and Bacillus circulans*. Other phosphorus solubilizing and mineralizing bacteria include various strains of *Azotobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Kushneria, Paenibacillus*(2), (6), *Ralstonia, Rhizobium, Rhodococcus, Serratia, Bradyrhizobium, Salmonella, Sinomonas, and Thiobacillus* (2), (6). The microbial fungi that function similarly include strains of *Achrothcium, Alternaria, Arthrobotrys, Aspergillus, Cephalosporium, Cladosporium, Curvularia, Cunninghamella, Chaetomium, Fusarium, Glomus, Helminthosporium, Micromonospora, Mortierella, Myrothecium, Oidiodendron, Paecilomyces, Penicillium, Phoma, Pichia fermentans, Populospora, Pythium, Rhizoctonia, Rhizopus, Saccharomyces, Schizosaccharomyces, Schwanniomyces, Sclerotium, Torula, Trichoderma, and Yarrowia* (2), (6). 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. There is no report for cyanobacteria (algae) about solubilizing ability (2). Different types of soil microorganisms are capable of solubilizing/mineralizing insoluble soil phosphate to release soluble P and making it available to plants. Phosphate solubilizing microorganisms improve both plant growth and crop yield. Therefore, inoculating seeds/crops/soil with PSM is a promising strategy to improve world food production without causing any environmental hazard (2). The same as their great significance in soil fertility improvement, phosphorus-solubilizing microorganisms have yet to replace conventional chemical fertilizers in commercial agriculture. A better understanding of recent developments in PSM functional diversity, colonizing ability, mode of actions and judicious application should facilitate their use as reliable components of sustainable agricultural systems (2), (6).

Importance of phosphorous in plant growth

Phosphorus (P) is considered to be one of the most essential macro-elements required for growth and development of plants. Phosphorus nutrition is associated with several key functions of the plants, which include development of roots, strengthening the stalks and stems, formation of flowers and seeds, crop maturity and quality of the production, nitrogen fixation in legumes and strengthening the plant against diseases(1). Phosphorus is an essential nutrient both as a part of several key plant structure compounds and as a catalysis in the conversion of numerous key biochemical reactions in plants. Phosphorus is noted especially for its role in capturing and converting the sun's energy into useful plant compounds. Phosphorus is a vital component of DNA, the genetic "memory unit" of all living things(1), (8). It is also a component of RNA, the compound that reads the DNA genetic code to build proteins and other compounds essential for plant structure, seed yield and genetic transfer. The structures of both DNA and RNA are linked together by phosphorus bonds(9). Phosphorus is a vital component of ATP, the "energy unit" of plants. ATP forms during photosynthesis, has phosphorus in its structure, and processes from the beginning of seedling growth through to the formation of grain and maturity. Phosphorus can naturally be found in diverse forms in the soil solution. They can broadly be categorized as insoluble inorganic phosphorus and insoluble organic phosphorus. However, due to low solubility and fixation in soils, only a small fraction of phosphorus exists in soil solution (1 ppm or 0.1%), is readily available to plants(1), (9).

Plants deficient in phosphorus are stunted in growth and often have an abnormal dark-green color. Sugars can accumulate and cause anthocyanin pigments to develop, producing a reddish-purple color. This can sometimes be seen in early spring on low phosphorus sites. These symptoms usually only persist on extremely low phosphorus soils. It should be noted that these are severe phosphorus deficiency symptoms and crops may respond well to phosphorus fertilization without showing characteristic deficiencies. In addition, the reddish-purple color does not always indicate phosphorus deficiency but may be a normal plant characteristic. Red coloring may be induced by other factors such as insect damage which causes interruption of sugar transport to the grain. Phosphorus deficiencies may even look somewhat similar to nitrogen deficiency when plants are small. Yellow, unthrifty plants may be phosphorus deficient due to cold temperatures which affect root extension and soil phosphorus uptake. When the soil warms, deficiencies may disappear. In wheat, a very typical deficiency symptom is delayed maturity, which is often observed on eroded hillsides where soil phosphorus is low(10).

Phosphorus is often recommended as a row-applied starter fertilizer for increasing early growth. University of Nebraska starter fertilizer studies conducted in the 1980s showed early growth response to phosphorus in less than 40 percent of the test fields (Penas, 1989). Starter applications may increase early growth even if phosphorus does not increase grain yield. Producers need to carefully evaluate cosmetic effects of fertilizer application versus increased profits from yield increases.

When compared with other nutrients like nitrogen and potassium diagnosing deficiency of phosphorous is most difficult than others. Because, crops usually display no obvious symptoms of phosphorus deficiency other than a general stunting of the plant during early growth. At the time when plants shows colour change because of phosphorous deficiency, the problem will be beyond diagnose. Some crops, such as corn, tend to show an abnormal discoloration when phosphorus is deficient. The plants are usually dark bluish-green in color with leaves and stem becoming purplish. Phosphorus is highly mobile in plants, and when deficient, it may be translocated from old plant tissue to young, actively growing areas. Consequently, early vegetative responses to phosphorus are often observed. As a plant matures, phosphorus is translocated into the fruiting areas of the plant, where high-energy requirements are needed for the formation of seeds and fruit. Phosphorus deficiencies late in the growing season affect both seed development and normal crop maturity. The percentage of the total amount of each nutrient taken up is higher for phosphorus late in the growing season than for either nitrogen or potassium(11).

Sources of Phosphate Solubilizing Microorganisms

The soil is a habitat for a vast, complex and interactive community of naturally occurring soil organisms, whose activities largely determine the physico-chemical properties of the soil and consequently promote the growth of the crop plants. From seed germination until a plant reaches maturity, it lives in close association with soil organisms(4). Fungi are the important components of soil microbes typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. Fungi have been reported to have greater ability to solubilize insoluble phosphate than bacteria. A wide range of soil fungi are reported to solubilize insoluble phosphorous such as *Aspergillus niger* and *Penicillium sp.* which are the most common fungi capable of phosphate solubilization (12).

Inoculation of Phosphate Solubilizing Fungi to Seeds

Most agricultural soils contain large reserves of phosphorus (P), a considerable part of which accumulates as a consequence of regular applications of P fertilizers. However, a greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants. Therefore, inoculating fungal strains having potential to solubilize insoluble inorganic phosphates has crucial role. Fungal species such as *Aspergillus* sp. and *Penicillium* sp. are known for their potential to solubilize insoluble phosphate accumulated in the soil. Phosphate solubilizing microorganisms convert insoluble phosphates into soluble forms generally through the process of acidification, chelation and exchange reactions. Thus such microorganisms may not only compensate for higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to soil (13).

For application of phosphate solubilizing fungi to seeds, the seeds should be surface sterilized, rinsed with sterile water and dried. The surface disinfected seeds can be coated by soaking seeds in liquid culture medium of phosphate solubilizing fungi for 2 hr. While inoculation of the seed using plant and both *Aspergillus niger* + *Penicillium notatum* is recommended. Thus the application of P solubilizing fungi is recommended as a sustainable way for increasing crop yield(14).

Mechanisms of Phosphate Solubilization by Microorganisms

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(15).

Naturally occurring rhizospheric phosphorus solubilizing microorganism (PSM) dates back to 1903. Bacteria, fungi, actinomycetes and even algae plays important role in P solubilization. Bacteria are predominant amongst them and proved more effective in phosphorus solubilization than fungi(5). The other mechanism is the production of H_2S, which react with ferric phosphate to yield ferrous sulphate with concomitant release of phosphate(3). The mechanism of P solubilization that is employed mostly by soil microorganisms includes: (1) release of complex compounds e.g. organic acid anions, siderophores, protons, hydroxyl ions, CO_2, (2) liberation of extracellular enzymes or it also referred as biochemical P mineralization and (3) the release of P during the degradation of substrate. Thus, microorganisms have key role in the soil P cycle i.e. precipitation, sorption–desorption, and mineralization(5).

Organic Phosphate Solubilization

Phosphorus can be released in the soil from organic compounds by three groups of enzymes: (1) Nonspecific phosphatases, which leads dephosphorylation of phospho- ester or phosphoanhydride bonds in organic matter, (2) Phytases, which mostly release P which is intact in the form of phytic acid, and (3) Phosphonatases and C–P Lyases, the phosphonates degrading enzyme enzymes that perform C–P cleavage in organo-phosphonates. Availability of organic phosphate compounds for plant nutrition could be a limitation because as phosphorous is highly reactive it will interact with other metallic elements that are present in the soil in the rhizospheric area and becomes unavailable to plants which retard the plant growth and subsequently crop yield. Therefore, the capability of enzymes to perform the desired function in the rhizosphere is a crucial aspect for their effectiveness in plant nutrition (5).

Inorganic phosphate solubilization

Microorganism plays an important role in P solubilization through secretion of organic acid production either by: (i) lowering the pH, or (ii) through chelation reaction of cations bound to P (iii) by competing with P for adsorption sites on the soil (5), (16). The lowering in pH of the medium suggests the secretion of organic acids by the P- solubilizing microorganisms via direct oxidation pathway that occurs on the outer face of the cytoplasmic membrane. When P is applied to soil it get interact with other metallic elements such as Fe, Al and Ca ions which makes the P unavailable to plants through the formation of ferrous phosphate, aluminium phosphate, calcium phosphate etc. and the release of organic acids by PSM leads the chelation reaction and because of this the bound P to other metallic elements get freed and becomes available to plants(1), (17). The prominent acids that are released by PSM in the solubilization of insoluble P are gluconic acid, oxalic acid, citric acid, lactic acid, tartaric acid and aspartic acid etc. (5).

Another mechanism is the production of H2S, which react with ferric phosphate to yield ferrous sulphate with the release of phosphate. It could be because of the activity of PSM occurs as a consequence of microbial sulphur oxidation, nitrate production and CO_2 formation. These processes ultimately leads the formation of inorganic acids like sulphuric acid (5), (18).

Contribution of Phosphate Solubilizing Microorganisms in Agriculture

There are several reports on plant growth promotion by bacteria that have the ability of solubilize inorganic and/or organic P from soil after their inoculation in soil or plant seeds. It was reported that a strain of *Burkholderia cepacia*, commercially used as biofertilizer in Cuba which display significant mineral phosphate solubilization and moderate phosphatase activity, also improve the yield of tomato, potato, onion, banana, coffee etc. Inoculation with two strains of *Rhizobium leguminosarum* selected for their P solubilization ability has been shown to improve root colonization and growth promotion and to increase significantly the P concentration in lettuce and maize. Also a strain of *Pseudomonas putida* stimulate the growth of roots and shoots and increased 32P-labeled phosphate uptake in canola. Co-inoculation of *Pseudomonas striata* and *Bacillus polymyxa* strains showing phosphate solubalizing activity, with a strain of *Azospirillium brasilense*, resulted in significant increase in grain and dry matter yields, with a concomitant increase in N and P uptake. Several studies have shown that PSB interacts with the vesicular arbuscular mycorrhizae (VAM) by releasing phosphate ions in the soil, which causes synergistic interaction that allows for better exploitation of poorly soluble P sources (5).

Current strategy is to maintain and improve agricultural productivity exclusively via the use of chemical fertilizers. Although the use of chemical fertilizers is credited with nearly fifty percent increase in agricultural production but they are closely associated with environmental pollution and health hazards (4), (19). Many synthetic fertilizers contain acids, such as sulfuric acid and hydrochloric acid, which tend to increase the acidity of the soil, reduce the soil's beneficial organism population and interfere with plant growth. Generally, healthy soil contains enough nitrogen-fixing bacteria to fix sufficient atmospheric nitrogen to supply the needs of growing plants. However, continued use of chemical fertilizers may destroy these nitrogen-fixing bacteria. Furthermore, chemical fertilizers may affect plant health. For example, citrus trees tend to yield fruits that are lower in vitamin C when treated with synthetic fertilizer. Lack of trace elements in soil regularly dosed with chemical fertilizers is not uncommon. This lack of vital micronutrients can generally be attributed to the use of chemical fertilizers. On the other hand biofertilizer adds nutrients to soil. Environmentally friendly biotechnological approaches offer alternatives to chemical fertilizers. Given the negative environmental impacts of chemical fertilizers and their increasing costs, the use of PSM is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture(19) (Fig. 1–4).

Figure.1 Phosphate Solubilizing Microorganism (PSM) (7)

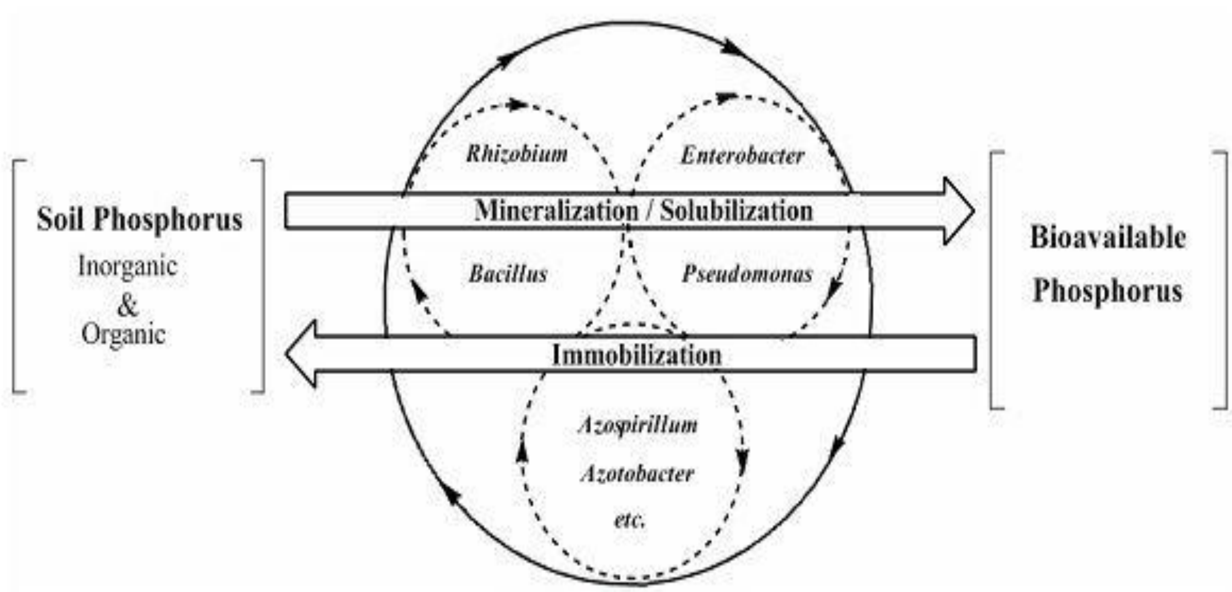


Figure.2 Inorganic and organic P solubilization pathways(7)

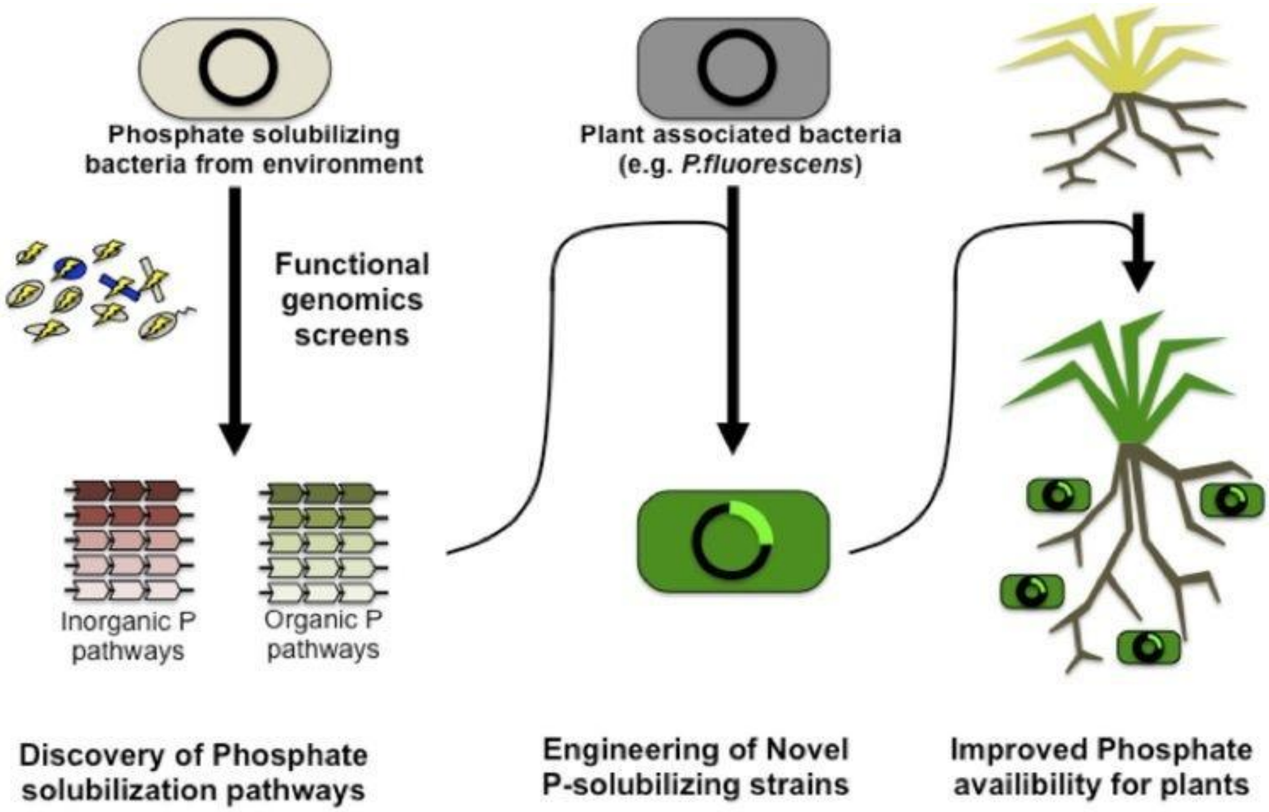


Figure.3 Contribution of PSM in plant growth (7)

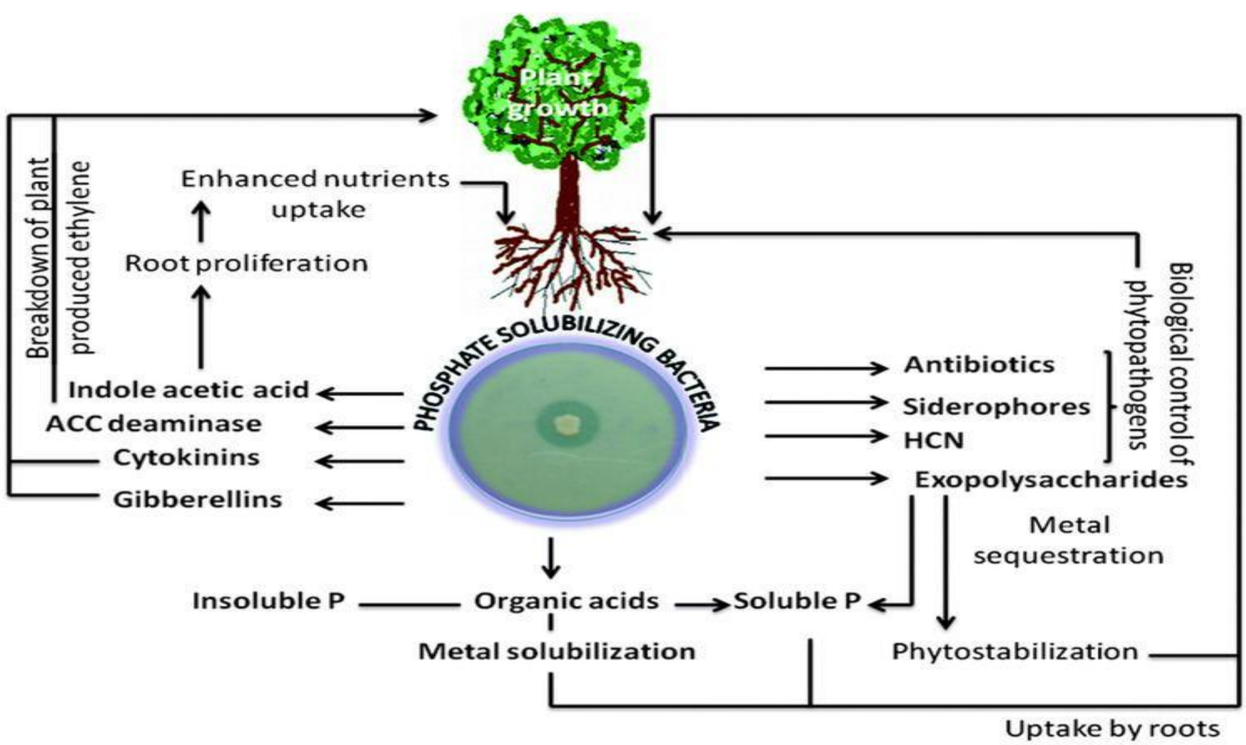
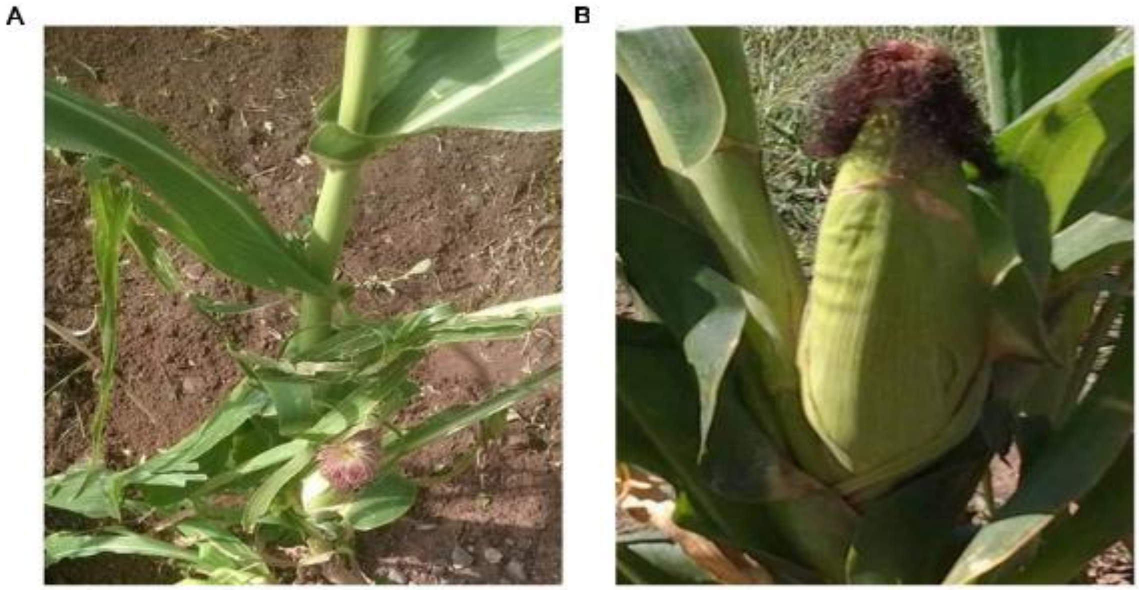


Figure.4 Effect of biofertilzers on plant growth: A) Maize not inoculated with PSM, B) Maize inoculated with PSM(2)



Many PSM inoculation studies have shown both improved plant yield and increased phosphorus uptake both in pot experiments and under field conditions. In a pot experiment where *Aspergillus niger* was used as a biofertilizer (using wheat husks with 20% perlite as carrier material) the soil colonization rate was 5.6 × 106 spores g−1 soil. The benefits of adopting microbial management of the rhizosphere for sustainable agriculture production includes enhancing the bioavailability of phosphate to crops, stimulated roots and shoots growth, improved root and shoot length, and increased fresh and dry shoot weights, P-labeled phosphate uptake, and significant improvement of grain and dry matter yields (2).

Conclusion

Phosphate solubilizing microorganisms convert insoluble phosphates into soluble forms generally through the process of acidification, chelation and exchange reactions. Current strategy is to maintain and improve agricultural productivity exclusively via the use of chemical fertilizers. Although the use of chemical fertilizers is credited with nearly fifty percent increase in agricultural production but they are closely associated with environmental pollution and health hazards. Microorganism plays an important role in P solubilization through secretion of organic acid production either by: (i) lowering the pH, or (ii) through chelation reaction of cations bound to P (iii) by competing with P for adsorption sites on the soil. Many species of microorganisms including bacterial species, fungal species, actinomycetes, and algae exhibit phosphate solubilization and mineralization ability. Accumulated phosphates in agricultural soils are sufficient to sustain maximum crop yields worldwide for about 100 years. Using phosphate solubilizing microorganisms to increase the yield of the crop is very advanced and important naturally available mechanism. Because, using PSM will not affect the soil in any direction. Most agricultural soils contain large reserves of phosphorus (P), a considerable part of which accumulates as a consequence of regular applications of P fertilizers. However, a greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants. Therefore, inoculating microbial strains having potential to solubilize insoluble inorganic phosphates has vital role. While application of phosphate solubilizing microbes to seeds, the seeds should be surface sterilized, rinsed with sterile water and dried. The surface disinfected seeds can be coated by soaking seeds in liquid culture medium of phosphate solubilizing microbes for 2 hr.

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How to cite this article:

Chala Dandessa and Ketema Bacha. 2018. Review on Role of Phosphate Solubilizing Microorganisms in Sustainable Agriculture. *Int.J.Curr.Res.Aca.Rev*. 6(11), 48-55.
doi: https://doi.org/10.20546/ijcrar.2018.611.006