

# Phosphorus Availability In Soil And Uptake By Maize From Rock Phosphate Inoculated With PGPR: A Review

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# Abstract

Plant growth promoting rhizobacteria (PGPR) are introduced and used in agro-ecosystems to improve plant-microbe interactions, which can affect ecosystem sustainability, agricultural productivity, and environmental quality. Microbial inoculants are being explored internationally to make the important ingredient for plant bioavailability under the current scenario of P restrictions to crop plants. Because P can be found in both organic and inorganic forms in soils, researchers should look for microbes that can solubilize both pools of P at the same time. Phosphorus

(P) is one of the most critical minerals for plant growth, and it ranks high among the soil macronutrients. Phosphate fertilisers are frequently used to compensate for soil P shortage. P deficit in soils is caused by lower total P levels in the soil and the inability to fix additional P from chemical fertilisers and other organic sources such as manure. The plant's response to P stress, or even when it is present in sufficient amounts, is relatively modest. The availability of P is limited by its solubilization, as it is fixed in both acidic and alkaline soils. Only phosphate-solubilizing bacteria can dissolve soil-fixed P. (PSMs). According to the literature examined, bacteria that exhibit numerous plant growth boosting properties rather than a single attribute should be picked during screening and selection procedures. Phosphatase activity, plant growth regulator production, and/or ACC-deaminase activity are examples of numerous characteristics. This method of identifying effective PGPR for enhancing nutrient availability for plant uptake could be more effective .

### Keywords: Phosphorus, PGPR, Maize,

## 1. Introduction

Maize (Zea mays L.) is a vital food and feed crop of the world and is often called as "the king of grain crops". It belongs to the family Poaceae or Gramineae. It ranks third most important cereal crop after wheat and rice in the world (Rebi, A, et al.2020). Besides being an important food grain for human consumption, maize has also become a major component of livestock and poultry feed (Witt and Pasuguin, 2007). Maize production in Pakistan during 2012-13 was about 4220 thousand tones which increased up to 4527 thousand tons in 2013-14 from total area of 1117 thousand hacters (Anonymous, 2014). As compared to other maize producing countries the average yield is less in Pakistan due to several factors and phosphorus availability is one of them. Phosphorus is very important for improving maize growth and yield. Phosphorus is a key nutrient element of a plant, next only to nitrogen and grouped as a major plant nutrient along with nitrogen and potassium. Its contribution in plant dry weight is about 0.2%. It has a fundamental role to play in plant growth and development. Different features of plant development like photosynthesis, formation of nucleus, consumption of sugars and starch, fat and albumin, cell division and cell organization and the transmit of heredity material are associated with phosphorus (Lal, 2002). The production of nucleic acid molecules (DNA and RNA) depends on phosphorus. Phosphorus is a fundamental constituent of energy rich biological molecule adenosine triphosphate (ATP) and also as hydrophilic phosphate groups of phospholipids which are important constituents of cell membranes (Marschner, 1990). For optimal growth, the phosphorus requirement ranges from 0.3-0.5% of plant dry weight. In plants, retarded growth followed by reddish coloration is mainly due to the deficiency of phosphorus. Under limited supply of P, the plant mostly appears as darker green in color. The plants deficient in P have much lower photosynthetic rate resulting in reduced metabolic processes. But unfortunately under semiarid conditions, plants are not able to get the required P due to high soil pH and low organic matter. Under semiarid conditions, the applied P-fertilizer is converted into immobile form due to calcareous soils under semiarid climate. About 30 to 40% maize yield is reduced due to the unavailability of P and lower P use efficiency. Some plant growth promoting rhizobacteria (PGPR) having trait P solubilization that are known as phosphate solubilizing bacteria. These bacteria have the ability to solubilize the fixed soil P by releasing of organic acid, which convert the insoluble P to soluble form by increasing the P availability to plants under semi-arid condition which is very important for increasing yield and profitability of maize. The objectives of this study is to find the effect of phosphorus on uptake in maize plant with PGPR

## 1.1. Phosphorus as a major plant nutrient

Phosphorus is an indispensable macronutrient which plays a pivotal role in the growth and development of crops. It is considered as the second most limiting nutrient after nitrogen in most of the soils and is the world's second highest chemical fertilizer input in agriculture. But in most soils its content is about 0.05% of which only 0.1% is available to plant (Scheffer et al., 1998). It has been reported that soils from arid and semi-arid regions of the world are very poor in available phosphorus ranging from 80-90% (Sander, 1986; Memon et al., 1992).

Although agricultural soils are rich in total P, yet it is unavailable to plants and is considered as a limiting factor for plant growth. The efficiency of phosphatic fertilizer is usually low due to the formation of insoluble complexes in soils (Rengel and Marschner., 2005). Of the total P in soils, organic P is present in large amount. The mineralization of organic P carried out by phosphatase enzymes releases orthophosphate ions in soils. Soil microorganisms are the major source of such enzymes. Besides conventional mineral phosphate fertilization, microbial P solubilization could help to improve the availability of P. Application of bacteria having P-solubilizing activity directly promotes uptake of P while some microorganisms with their 1-aminocyclopropane-1-carboxylate (ACC) activity or auxin production capability can further enhance P attainment by plant indirectly through better root system. This review covers the issues regarding P availability and the role that microorganisms play through unlike mechanisms to replenish this nutrient in soils.

Phosphorus exists in soils as organic and inorganic forms. Organic matter mostly comprises of P as phospholipids, nucleotides and inositol phosphate (Anderson, 1975; Halstead and Mackercher, 1975). It is likely that soil organic P (SOP) does a major job in P nutrition of crops mainly in calcareous soils of high P fixing capacity (Tarafdar and Claasson, 1988).

The optimal development of crops demands high, often costly, input of P fertilizer. Current concepts in sustainability involve application of alternative strategies based on the use of less expensive natural sources of plant nutrients like rock phosphate (RP). The beneficial effect of rock phosphate has made this material an attractive component for management in agriculture (Rajan et al., 1996). One traditional method of increasing P-availability is the acidulation of RP with small amounts of  $H_2SO_4$  or  $H_3PO_4$  to produce partially acidulated RP (Rajan and Watkinson, 1993). But this is uneconomical and environmentally nonviable. Most of the soils contain phosphorus in the form of insoluble compounds which is unavailable to plants. It is a common practice that to replenish soil nutrients, chemical fertilizers are used in bulk quantities which has resulted in high expenses and environmental contamination (Dai et al., 2004). Rock phosphate is an important raw material for phosphatic chemical fertilizers. (Isherwood., 1998 and Jasinski., 2006) warned that the world reserves of phosphate rock are becoming increasingly scarce and it is estimated that they would be exhausted within next 50-100 years, with a global peak in treatment of P reserves occurring by 2040. Furthermore, the quality of phosphate rock is decreasing and its cost is increasing (Cordell, 2008). In order to overcome these inefficiencies, microbial inoculants are now being investigated worldwide for their potential to mobilize unavailable organic and inorganic P sources for sustainable agriculture.

In soils, there are many bacteria associated with plant roots which are termed as rhizobacteria. Those rhizobacteria which help the plants in their growth and development, are frequently named as plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1989). These rhizobacteria affect plant growth and development directly and indirectly through their different mechanisms of action (Parsello-Cartieaux

et al., 2003; Mantelin and Touraine, 2004, Datta et al., 2011). The PGPR enhance the growth of plants directly by providing the plant with a compound which is synthesized by the bacterium or facilitation the uptake of plant nutrients such as Fe and P. The PGPR enhance plant growth indirectly through increased growth restricting circumstances (Glick et al., 1999). On the other hand, direct growth enhancement by PGPR commonly involves providing the plant with a compound which is synthesized by the bacterium or facilitating the uptake of plant nutrients such as P and Fe. Some of other direct ways through which these rhizobacteria could help in the propagation of their host plants which include; fixation of atmospheric nitrogen, solubilization of mineral nutrients such as Fe and P, production of siderophores which help in solubilizing Fe for plant uptake, production of plant growth regulators which can improve variety of stages of growth of plant, and synthesis of enzymes which may alter the growth and development of plants (Lambert and Joos, 1989; Patten and Glick, 1996; Glick et al, 1999). Several PGPR have been reported to improve growth of different crops e.g. canola, soybean, lentil, pea, wheat and radish (Glick et al., 1997; Timmusk et al., 1999; Salamone, 2000). The improvement of plant growth by PGPR in agricultural crops describes their potential to be used as biofertilizers for sustainable agriculture.

Biofertilizers or microbial inoculants are substances which consist living organisms which increase growth and productivity of crop plants (Rao and Dommergues, 1998). The ability of microorganisms to solubilize P is known to be one of the most important feature related to plant nutrition (Chen et al., 2006). Different bacterial species in association with plant rhizosphere have the ability to enhance phosphorus availability to plants either by solubilization of inorganic phosphates, thorough production of organic acids or by mineralization of organic phosphate by phosphatases (Rodriguez and Fraga, 1999). Such bacteria are commonly called as phosphate solubilizing bacteria (PSB) and are known to have probable implication as microbial inoculants for improving the growth and yield of plants (Vessey, 2003; Chen et al., 2006).

The production of plant hormones or phytohormones is also one of the direct mechanisms exhibited by plant growth promoting rhizobacteria (PGPR) for improving the growth of plants (Glick, 1995). Phytohormones are the organic substances other than plant nutrients or vitamins, which at enormously low concentration affect various physiological activities of growth and development in plants. These phytohormones include auxins, cytokinins, gibberellins, ethylene and abscisic acid. Auxins being a class of phytohormones are involved in the regulation of growth and development throughout the life cycle of plants. These are endogenously synthesized by plants and their exogenous application can also affect plant growth. It is well documented that several soil microbes are dynamically concerned in the synthesis of auxins in pure culture as well as in soil (Arshad and Frankenberger, 1998; Biswas et al., 2000; Asghar et al., 2004). This auxin production by the rhizobacteria is modified by various factors; the important one is rhizosphere difference of different crops/species.

Similarly, some plant growth promoting rhizobacteria with their ACC deaminase activity can improve phosphorus acquisition by plant indirectly by increasing root biomass. Ethylene being an important hormone is concerned with the regulation of various physiological processes in plants (Arshad and Frankenberger 2002; Owino et al., 2006). Under nutritional stress such as P deficiency, the level of ethylene increases which is considered inhibitory to plant growth especially for root growth. Naik et al. (2008) reported that some phosphate solubilizing bacteria also produce ACC-deaminase as a plant growth promoting enzyme. Soil bacteria containing ACC deaminase activity are helpful in increasing root elongation through lowering ethylene levels in plant roots by converting ACC into NH<sub>3</sub> and  $\alpha$ -ketobutyrate in plants (Penrose and Glick, 2003). It is likely that increased root growth could help in larger uptake of P

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and other nutrients for better plant growth, consequently, the bacteria having ACC-deaminase activity along with phosphatase enzymes or auxin production capability could be more effective in increasing the P availability to plants and ultimately increase the yield (Zahir et al., 2003).

## 1.2. Use of phosphatic fertilizers and the importance of phosphate rock (PR)

The majority (76-90%) of soluble P fertilizers used for agricultural production precipitates after application, by forming metal cation complexes (Khan et al., 2007). Because P chemical fertilizers are expensive, the cheaper direct use of PR with high reactivity is an interesting alternative (Babana and Antoun 2006; Smalberger et al., 2010). It is the cheapest P fertilizer but because of its low reactivity, acid pre-treatment is required to reach the effectiveness of other P fertilizers (Rajan et al., 1996). However, its reactivity also depends on the PR source, particle size, and soil conditions (Garth 1984).

Phosphate rock is generally applied in acid soils and can take a few years of annual application before it is as effective as superphosphate (Garth 1984; Ghani et al., 1994). One of the main obstacles to direct application of phosphate rocks (PRs) to soil is the failure of PRs to release P in sufficient quantity to support plant growth. The relative agronomic effectiveness (RAE) of the PR depends on soil characteristics and PR reactivity (Bolland and Gilkes, 1997; Smalberger et al., 2010).

Several alternatives for increasing PR reactivity have been tried; a) incorporation of additives, b) partial acidulation (Kpomblekou-A and Tabatabai, 2003), c) compaction of PR with water-soluble P fertilizers (Kpomblekou-A and Tabatabai 2003), and d) the use of phosphate- solubilizing bacteria (Vassileva et al., 1997). PSB are a very attractive approach for using PRs as fertilizer because they are able to solubilize P by excreting organic acids (Reyes et al., 2001).

Phosphorus (P) is an essential nutrient required for plant growth and development. However, a large proportion of the P present in soil is unavailable to plants. In calcareous soils the presence of Ca<sup>+2</sup> increases P precipitation (Tunesi et al., 1999) and in acid soils Al and Fe oxides are responsible for P fixation (Anderson et al., 1974). For this reason, large quantities of expensive phosphatic fertilizers are used to compensate for the limited availability of P, but this practice is not recommended due to potential environmental problems generated by runoff and erosion causing eutrophication (Sharpley et al., 1994). Direct application of phosphate rock (PR) can replace more expensive water soluble P fertilizers, especially in developing countries.

Certain soil microorganisms can help plants to acquire P through different mechanisms such as increasing root development by hormonal stimulation (i.e. root growth, branching, or root hair development), altering the soil P sorption equilibrium, and solubilizing and mineralizing P from less available forms of organic and inorganic P (Richardson and Simpson, 2011). It has been observed that plant inoculation with P-solubilizing bacteria (PSB) enhances plant growth by increasing the availability of already accumulated phosphates in soil and/or by mobilizing P from low-grade PR (Hameeda et al., 2008). Pseudomonas, Bacillus, Rhizobium and Bradyrhizobium strains (Halder et al., 1990) can mobilize P from low solubility P sources. Plant growth improvement by PSB inoculation has been observed in lettuce (Chabot et al., 1996), maize (Reyes et al., 2002), wheat (Babana and Antoun, 2006), alfalfa (Barea et al., 2002) and other crops.

#### 1.3. Role of microbes in phosphorus availability

For optimum plant growth, a sufficient supply of mineral nutrient is required. Glick (1995) is of view that the mobilization of mineral nutrient like phosphorus and iron in soil, by PGPR which makes these nutrients in more readily plant available forms should be employed as a mechanism for increased growth and development of plants. Although if soils contain an abundant amounts of an essential nutrients, yet the plant can exhibit deficiency symptoms owing to the non-availability of some of these essential nutrients in soil. Many researchers have reported that PGPR can induce growth of plants by enhancing solubilization (through the production of organic acids, exopolysaccharides, production of H<sup>+</sup> ion, and siderophores) and thus help plant to uptake mineral nutrients (Glick, 1995; Biswas et al., 2000b; Dazzo et al., 2000).

The main sources of phosphorus in soils include organic manures, rock phosphate and di-ammonium phosphate (DAP). On average basis, all the mineral nutrients other than P are found in mill molar quantities in soil solution, whereas P is found mostly in micro molar or in smaller amounts (Ozanne, 1980). Such low levels of P in soils are mainly attributed due to higher reactivity of soluble phosphorus with calcium (Ca), iron (Fe) or aluminium (AI) resulting in P precipitation in soils. Mostly inorganic P is associated with Fe and Al compounds in acidic soils (Sharpley et al., 1984) while in calcareous soils calcium phosphates are the principle form of inorganic phosphates.

In order to maximize crop productivity, the use of phosphatic fertilizer is a basic need. In general the efficiency of applied fertilizer is low due to formation of insoluble complexes with soil colloids (Dobermann et al., 1998; Vassilev and Vassileva, 2003). Phosphorus is solubilizes mainly through the mechanism of precipitation and adsorption on Ca, Fe, and Al etc. (Harris et al., 2006). Compared with the other major plant nutrient elements, P is considerably the least mobile and available to plants under most soil conditions. Several soils all over the world are lacking in available phosphorus as available P concentration is usually not greater than 10  $\mu$ M under a favorable pH of 6.5 even in well soil (Gyaneshwar et al., 2002). One of the major factors limiting growth and productivity of crop plants in many ecosystems of the world is related to phosphorus deficiency (Raghothama and Karthikeyan, 2005).

To overcome P deficiency in plants, PGPR containing phosphate solubilizing ability could play an important role in providing phosphate to plants in a more environmental friendly and on sustained basis. Plant growth promoting rhizobacteria containing phosphate solubilizing capability are also called as phosphate solubilizing bacteria (PSB) which are capable of growing on various P containing compounds. These PSB not only accumulate P, but also release a great part of soluble phosphate in soil for plant uptake. It has been found that PSB solubilize the low soluble calcium phosphate compounds through the production of phosphatases and make them available to plants (Rao, 2000).

The soil microorganisms are concerned with several processes which help in the exchange of soil P and thus are playing a significant part in the soil P cycle. Specifically, these microbes are helpful in releasing P both from inorganic and organic sources of soil P through their respective solubilizing and mineralizing abilities. In soil, the microbial biomass also contains a major portion of immobilized P which is potentially available to plants. In this way, soil microorganisms, are critical for the transferring and maintaining the P from inadequately available soil pools to plant available forms. The rhizosphere of plants is considered as the main place for such processes (Richardson, 2001). The activity of different phosphatases in the rhizosphere of maize, wheat and barley was evaluated by Burns (1982) and found that phosphatase activity was considerably higher at acidic and neutral soil pH in the inner rhizosphere of the plants. Among the acid phosphatase producing bacteria, strains from the genus Bacillus, Rhizobium, Citrobacter,

Enterobacter, Klebsiella, Proteus and Pseudomonas have been reported by Abd-Alla (1994); Thaller et al. (1995); Gügi et al., 1991 and also by Skrary and Cameron (1998). Different researchers have also revealed that some microorganisms containing phosphatase activity are also involved in the solubilization of inorganic form of phosphorus under P limitation. Natesan and Shanmugasundaram (1989) studied the in vitro solubilization potential by the cyanobacterium Anabaena ARM310. Under conditions of phosphate depletion, they found that cyanobacteria showed enhanced cell surface and intracellular alkaline phosphatase activity. It was also revealed that under phosphorus limitation in the media, alkaline phosphatase activity was very prominent. They suggested that solubilization of tri-calcium phosphate was brought by alkaline phosphatases secreted by cyanobacterium Anabaena ARM310.

# 1.3.1. Mechanisms of microbial phosphate solubilization and mineralization

Phosphorus exists as organic and inorganic forms in soil. The exact mechanism by which phosphate solubilizing bacteria (PSB) can increase growth of plants is still unclear, however, the accepted mechanism in mineral phosphate solubilization is considered to be due to organic acids production by these bacteria. On the other hand it is likely that phosphatases are involved in the mineralization of organic phosphorus in soil (Rodriguez and Fraga, 1999; Sarapatka, 2003). Rhizosphere microorganisms can help in mobilizing P from soils or low quality rock fertilizer through different mechanisms such as acidification to solubilize P from acid soluble soils and fertilizers, by releasing organic anions to mobilize P in hydrous oxide bound P or by releasing phosphatase enzymes for the mobilization of organic phosphorus (Trolove et al., 2003). Arun (2007) suggested two main schools of thought interpreting the mechanism of microbial P solubilization as follows; a) solubilization by production of organic acids, b) solubilization by production of phosphatase enzymes.

# 1.3.1.1. Microbial mediated inorganic phosphate solubilization

Although, agricultural soils are rich in total phosphorus, yet only a small proportion of it which is less than 1% is instantly available for uptake of plant (Russell, 1973). A very small portion of P in soil is found in the forms which are soluble in water. Nearly, 90% of the soil P is present in unavailable or fixed forms including primary minerals of phosphate, Ca, Fe, Al, phosphates fixed by colloidal oxides and silicate minerals and humus P. Plants take phosphorus mostly in the form of negatively charged orthophosphate ions (primary and secondary). Therefore, the pools of P which are soluble in soil water put a direct effect on growth of plants. Inorganic P can be mobilized to available form by the rhizobacteria (Laslo et al., 2012). In order to solubilize inorganic phosphates, P-solubilizing bacteria secrete different kinds of organic acids (i.e. Acetate, oxalate, tartrate, succinate, citrate, gluconate, ketogluconate and glycolate) and some of the organic acids which are mostly secreted by these microorganisms (lyamuremye and Dick, 1996; Puente et al., 2004). It has been reported that organic acids released by these microorganisms reduce soil pH resulting in enhanced availability of P for plants (Rodriguez and Fraga, 1999). Production of organic acids by phosphate solubilizing bacteria is well documented in literature and according to Illmer and Schinner (1992), the most frequently secreted organic acid, is gluconic acid which is known as the principle organic acid secreted by P-solubilizing bacteria for the solubilization of mineral phosphate. The second major organic acid found in phosphate solubilizing bacteriais 2-ketogluconic acid (Halder and Chakrabartty, 1993). Some strains of PSB have been reported to secrete a mixture of organic acids such as lactic,

isovaleric, isobutyric and acetic acids. Similarly, glycolic, oxalic, succinic and malonic acids have also been determined in phosphate solubilizing bacteria (Ilmer and Schinner, 1992).

On the basis of clear zone produced on Pikovskaya and Jensen agar media, Kumar and Narula (1999) tested different mutant phosphate solubilizing strains of Azotobacter chroococcum obtained from wheat rhizosphere to evaluate their capability to solubilize tricalcium phosphate (TCP) and mussoorie rock phosphate (MRP) along with production of auxin (as IAA equivalents). They found that the mutants of A. chroococum strain P-4 had higher phosphate solubilization (TCP 1.52  $\mu$ g mL<sup>-1</sup> and MRP 0.19  $\mu$ g mL<sup>-1</sup>) and they also observed that mutants of A. chroococum strain P-4 also produced maximum amount of auxin in the cultured media. Nautiyal (1999) affirmed that organic acid production is a key mechanism in 'P' solubilization but not the only mechanism.

# 1.3.2. Role of phosphate solubilizing bacteria in crop production

Significant increases in growth and yield of agriculturally important crops in response to inoculation with plant growth promoting rhizobacteria have been reported (Chen et al., 1994; Asghar et al., 2002). Similarly, several studies have documented that PSB enhanced the growth and yield of inoculated crop plants. The PSB inoculants gave higher yields up to 20% in case of maize and lettuce (Chabot et al., 1993). Similarly, significant increases in the growth and yield of other agriculturally important crops have been reported in response to the inoculation with PSB (Biswas et al., 2000; Hilali et al., 2001; Asghar et al., 2002).

It is well established that under in vitro conditions, P-solubilizing bacteria are involved in the solubilization of different kinds of naturally occurring phosphatic compounds. However, the variation in the performance of these PSB under field conditions had greatly hindered their extensive use in agricultural sustainability (Saghir et al., 2007). In spite of their variable performance, these PSB are being used in agronomic practices for improving crop production and maintaining soil health. The application of microbes having phosphate solubilizing ability as microbial inoculants or bio-fertilizers improves plant P uptake and crop yield. One of the most important bacterial physiological traits in soil biogeochemical cycles is considered to be solubilization and mineralization of phosphorus by P-solubilizing bacteria and thus improving plant growth (Jeffries et al., 2003).

Phosphate solubilizing PGPR can enhance growth of non-legume as well (Chabot et al., 1996). Addition of rock phosphate and microbial inoculation increased biomass production and accumulation of P in alfalfa (Barea et al., 2002). An increase in phosphorus availability to plants by the inoculation of PSB has been reported priorly in pot and under field conditions (Chabot et al., 1996; Zaidi et al., 2003). Gaind and Gaur (2004) found that use of rock phosphate, coupled with PSB, produced results comparable to superphosphate + PSB inoculants.

### 1.3.3. Combined use of P-solubilizing bacteria and rock phosphate for P nutrition of crops

Soil microorganisms have enormous potential in providing soil phosphates for plant growth. Rock phosphate is a less expensive and insoluble source of plant nutrients, which can serve as an alternative source of P in developing countries like Pakistan. Low grade rock phosphate occurs on every continent which is generally unsuitable for soluble fertilizer processes. It is less effective than single super phosphate (SSP), triple super phosphate (TSP) or diammonium phosphate (DAP) regarding its direct application. Availability of RP can be improved by using microbial processes (Zapata et al., 1995). PSB can help in

enhancing the accessibility of fixed or accumulated phosphates for plant growth through the process of solubilization (Chabot et al., 2006; Khan et al., 2007). Use of rock phosphate along with microbial inoculation enhanced biomass production and P buildup in alfalfa (Barera et al., 2002).

Sharma (2003) conducted a field study to evaluate the effect of P application on grain and straw yields, NPK uptake of rice-wheat cropping system and organic C, available P and K content of soil with and without inoculation. Application of uninoculated Mussoorie rock phosphate showed non-significant effect on yield parameters of rice. However, inoculation with P-solubilizing bacteria increased grain yield from 0.9-1.8 tons ha<sup>-1</sup>, straw yield from 0.8-2.1 tons ha<sup>-1</sup>, N uptake from 18-38 kg ha<sup>-1</sup>, P uptake from 2.7-6.6 kg ha<sup>-1</sup> and K uptake from 16-41 kg ha<sup>-1</sup> in rice-wheat cropping system. These increases were similar as obtained with DAP which increased available P in soil from 3-4.7 kg ha<sup>-1</sup>. Sundra et al. (2002) studied the influence of P solubilizing bacteria Bacillus megatherium var. phosphoticum with and without different quantities of phosphate fertilizers on changes in soil available P, growth and yield of sugarcane. The results revealed that PSB population and soil available P increased due to the application of PSB. Similarly, tillering, stalk population and stalk yield increased leading to 12.6% increase in cane yield in comparison to uninoculated control. Application of PSB resulted in a 25% reduction of phosphatic fertilizers. Additionally when P fertilizer and RP were applied (50: 50) along with PSB cane yield and sugar yield with 100% P as SSP alone, resulting in cost effective solution to P application. Similarly, Sharma and Prasad (2003) studied the effect of PSB Pseudomonas striata and residue incorporation on the efficacy of DAP and MRP (Mussorie rock phosphate) in rice-wheat rotation. The results revealed non-significant effect of MRP application on grain and straw yield as well as P uptake of both the crops. Results were strengthened by applying crop residue along with MRP + PSB making it at par with DAP. Furthermore, available P in soil after three year was more promising where MRP + PSB was applied along with residue incorporation than with DAP alone. Goyal et al. (2010) tested PSB strain P-36 for solubilization of rock phosphate to be used as P source for pearl millet-wheat cropping system with and without FYM and their results revealed that seed inoculation and RP without FYM didn't show an significant increase in bacterial count as well as crop yields but seed inoculation as well as application of 2 t ha<sup>-1</sup> FYM along with RP resulted in beneficial results regarding bacterial count and crop yield of both the crops under field conditions.

## 1.4. Future prospectus of P-solubilizing bacteria

P-solubilizing bacteria are an integral component of soil microbial community and play a vital role in P cycle in soil rendering the unavailable P to plants. These have enormous potential for making use of fixed P in the soil particularly in soils with low P availability. However, despite considerable promise microbial products for P mobilization have not had major application to broad-acre farming systems. Because phosphate solubilization by bacteria is a complex phenomenon affected by many factors, such as PSB used, nutritional status of soil and environmental factors. The success of biofertilizer program ultimately depends on aspects such as cost effective ratio, widespread applicability of specific strain, development of practical delivery systems and sustained positive results.

Although potential clearly exists for developing such inoculants, but their widespread application remains limited by a poor understanding of microbial ecology and population dynamics in soil, and by inconsistent performance over a range of environments. Hence the formulation of PSB inoculation with a reliable and consistent effect, under field conditions is still a bottleneck for their wider use. Therefore, more research is needed to explore the impact of PSB in affecting the various physiological, biochemical

and molecular events governing the stimulation of growth by these microbes in the plants. Hence, it needs further studies to understand the characteristics and mechanisms of phosphate solubilization by PSB. To conclude, the efforts should be made to identify, screen and characterize more PSB for their ultimate application under field conditions. So that, the successful implementation of PSB to better exploit soil P resources can be an alternative sustainable strategy for management of soil to optimize P bioavailability. The use of PSB has considerable promise for the future as a best management practice for soil to optimize P fertilization to meet the demands of crop production with minimal soil impacts. The use of PSB as inoculants becomes important to the sustainable management of soil and likewise contributes to environmental and economic stability

# 1.5. Concluding remarks

Under current scenario of P limitations to the crop plants, the microbial inoculants are being investigated worldwide to make the essential element for plant bioavailable. As P is found both as organic and inorganic forms in soils, therefore efforts should be made to explore those microorganisms which could solubilize these two pools of P, simultaneously. From the literature reviewed it can be emphasized that during screening and selection strategies, those bacteria should be selected which show multiple plant growth promoting traits rather than a single trait. These multiple traits could be included as phosphatase activity, plant growth regulators production and/or ACC-deaminase activity. Such approach could be more effective for selecting effective PGPR for improving the nutrient availability for plant uptake and consequently to increase the growth and yield of crop plants under reduced nutrient availability situations.

# References

- Abd-Alla, M.H. 1994. Phosphatases and the utilization of organic P by Rhizobium leguminosarum biovarviceae. Lett. Appl. Microbiol., 18: 294-296.
- Anderson, G., E.G. Williams, J.O. Moir. 1974. Comparison of sorption of inorganic orthophosphate and inositol hexaphosphate by 6 acid soils. J. Soil Sci., 25: 51–62.
- Asghar, H.N., Z.A. Zahir., M. Arshad and A. Khaliq. 2002. Relationship between in vitroproduction of auxins by rhizobacteria and their growth promoting activities in Brassica juncea L. Biol. Fert. Soils., 35: 231-237.
- Asghar, H.N., Z.A. Zahir., M. Arshad. 2004. Screening rhizobacteria for improving the growth, yield, and oil content of canola (Brassica napus L). Aust. J. Agric. Res., 55: 187-194.
- Arshad, M., W.T. Frankenberger. 1998. Plant growth-regulating substances in the rhizosphere: Microbial production and functions. Adv. Agron., 62: 46-151.
- Arun, K.S. 2007. Bio-fertilizers for sustainable agriculture. Sixth edition, Agribios publ.Jodhpur, India.
- Aziz-Qureshi, A and G. Narayanasamy. 1980. Dried effect of rock phosphates and phosphate solubilizers on soybean growth in a typic ustochrept. J. Ind Soci. Soil Sci. 47: 475-478.

- Babana, A.H, H. Antoun. 2006. Effect of Tilemsi phosphate rock solubilizing microorganisms on phosphorus uptake and yield of field grown wheat (Triticum aestivum L.) in Mali. Plant Soil., 287: 51–58.
- Barea, J.M., M. Toro, M.O. Orozco, E. Campos and R. Azcon. 2002b. The application of isotopic 32P and 15N-dilution techniques to evaluate the interactive effect of phosphate solubilizing rhizobacteria, mycorrhizal fungi and Rhizobium to 123 improve the agronomic efficiency of rock phosphate for legume crops. Nutr.Cycling Agroecosyst., 63: 35-42.
- Biswas, J.C., J.K. Ladha., F.B. Dazzo. 2000. Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Sci. Soc. Am. J. 64: 1644-1650.
- Burns, R.G. 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol. Biochem., 14: 423-427.
- Chabot, R., H. Anton and M.P. Cescas 1996. Growth promotion of maize and lettuce byphosphatesolubilizing Rhizobium leguminosarumbiovar. phaseoli. Plant Soil., 184:311-321.
- Chanway, C.P., Hynes, R.K. and Nelson, L.M. 1989. Plant growth promoting rhizobacteria: Effects on growth and nitrogen fixation of lentils and pea. Soil Biol. Biochem., 21: 511-517.
- Chaudhary, A.R. 1983. Agronomy in "Maize in Pakistan" Punjab Agriculture Coordination board Univ. Agri. Faisalabad, Pakistan.
- Cheuk, W., Lo.K.V., Branion, R. M., Fraser, B. (2003). Benefits of sustainable waste management in the vegetable greenhouse industry. J. Environ. Sci. Health B., 38:855–863. 10.1081/PFC-120025565.
- Cooper, R. 1959. Bacterial fertilizers in the Soviet Union. Soil Ferti., 22: 327-333.
- Cordell, D. 2008, 8 reasons why we need to rethink the management of phosphorus resources in the global food system, Information Sheet 1, Global Phosphorus Research Initiative (GPRI).
- Dai, A., K.E. Trenberth, and T. Qian. 2004, A global dataset of Palmer Drought Severity Index for 1870-2002: Relationship with soil moisture and effects of surface warming, J. of Hydrometeorology., 5:1117-1130.
- Datta, S., C.M. Kim., M. Pernas., N.D. Pires, H. Proust., T. Tam., P. Vijayakumar., and L. Dolan. 2011. Root hairs: development, growth and evolution at the plant-soil interface. Plant Soil., 346:1-14.
- Dazzo, F.B., Y.G. Yanni, R. Rizk., F.J. de Bruijn., J. Rademaker., A. Squartini., V. Corich., P.E. Mateos., E. Martínez-Molina., J.C. Velázquez., R.J. Biswas., J.K. Hernandez., J. Ladha., Hill., J. Weinma, Hartmann., M. Umali-Garcia and M.L. Izaguirre-Mayoral. 2000. Progress in multinational collaborative studies on the beneficial association between Rhizobium leguminosarumbv. Trifoliiand rice. In: Ladha J.K. and P.M. Reddy (eds.), The quest for nitrogen fixation in rice. IRRI, Los Banos, Philippines. p., 167-189.
- Dobermann, A., K.G. Cassman, C.P. Mamaril and J.E. Sheehy. 1998. Management of phosphorus, potassium, and sulfur in intensive, irrigated lowland rice. Field Crops Res., 56: 113-138.
- Gaur, A.C. and Geeta. 1983. Role of integrated plant nutrient systems in sustainable and environmentally sound agricultural development: RAPA Publication: 1993/13, FAO, Bangkok. pp., 110-130.

- Glick, B.R., D.M. Karaturovic and P.C. Newell. 1995. A novel procedure for rapid isolation of plant growth promoting pseudomonas. Can. J. Microbiol., 41: 533-536.
- Glick, B.R., C. Liu., S. Ghosh and E. B. Dumbroff. 1997. Early development of canola seedlings in the presence of the plant growth promoting rhizobacterium Pseudomonas putida GR 12-2. Soil Biol. Biochem., 29: 1233-1239.
- Glick, B.R., Patten, C.L., Holguin, G. and Penrose, D.M. 1999. Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London, United Kingdom, pp., 267.
- Gügi, B., N. Orange, F. Hellio, J.F. Burini, C. Guillou, F. Leriche and J.F. Guespin-Michel.1991. Effect of growth temperature on several exported enzyme activities in the psychrotropic bacterium Pseudomonas fluorescens. J. Bacteriol., 173: 3814-20.
- Goyal, S.M. Walia., Chand and R.C. Anand. 2010. Mobilization of phosphorus from rock phosphate by using phosphate solubilizing bacteria and FYM in pearl millet and wheat. Environ. Ecol. Vol., 28: 581-583.
- Halder, A.K. Mishra, A.K. Bhattacharya., P.K. Chakrabartty 1990. Solubilization of rock phosphate by Rhizobium and Bradyrhizobium. J. Gen. Appl. Microbiol., 36: 81-92.
- Halder, A.K. and P.K. Chakrabartty. 1993. Solubilization of inorganic phosphate by Rhizobium. Folia. Microbiol. 38: 325-30.
- Hameeda, B., G. Harini., O.P. Rupela., S.P. Wani and G. Reddy. 2008. Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. Microbiol. Res., 163: 234-242.
- Hameeda, B., G. Harini, O.P. Rupela, S.P. Wani and G. Reddy. 2008. Growth promotion of maize by phosphate solubilizing bacteria isolated from compost and microfauna. Microbiology Res., 163: 234-242.
- Harris, J.N., P.B. New and P.M. Martin. 2006. Laboratory tests can predict beneficial effects of phosphatesolubilizing bacteria on plants. Soil Biol. Biochem., 38: 1521-1526.
- Iyamuremye, F., Dick, R.P. and J. Baham. 1996. Organic amendments and phosphorus dynamics: II. Organic amendments and P fractions. Soil Sci., 161: 436-443.
- Jeffries, S., S. Gianinazzi, S. Perotto, K. Turnau, J.M. Barea. 2003. The contribution of arbuscular mucorhizal fungi in sustainable maintenance of plant health and soil fertility. Biol. Fertil., Soils 37: 1-16.
- Jasinski, S. M. 2006, Phosphate Rock, Statistics and Information, US Geological Survey.
- Khan, K.S. and R.G. Joergensen. 2006. Microbial C, N, and P relationships in moisture-stressed soils of Potohar, Pakistan. J. Plant Nutr. Soil Sci., 169: 494-500.
- Khan, M.S., A. Zaidi and P.A. Wani. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture. A Review. Agron. Sustain. Dev., 27: 29-43.
- Kushwaha, H.S. 2007. Response of chickpea to biofertilizers, nitrogen and phosphorus fertilization under rainfed environment. J. Food Legumes., 20:179–181.

- Kloepper, J.W., R. Lifshitz and R.M. Zablotowicz. 1989. Free living bacterial inocula for enhancing crop productivity. Trends in Biotech. 7: 39-44.
- Kumar, V. and N. Narula. 1999. Solubilization of inorganic phosphates and growth emergence of wheat as affected by Azotobacter chroococcum mutants. Biol. Fertil.Soils., 28: 301-305.
- Lal, L. 2002. Phosphate mineralizing and solubilizing microorganisms. p. 224. In: Phosphatic Biofertilizers. Agrotech Publ. Academy, Udaipur, India.
- Lambert, B., H. Joos. 1989. Fundamental aspects of rhizobacterial plant growth promotion research. Trends Biotechnol. 7: 215-219.
- Laslo, E., E. Gyorgy., G. Mara., E. Tamas., B. Abraham and S. Lanyi. 2012. Screening of plant growth promoting rhizobacteria as potential microbial inoculants. Crop Protec. 40:43-48.
- Mantelin, S., B. Touraine (2004). Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J. Exp. Bot., 55 27–34 10.1093/jxb/erh010
- Naik, P.R., G. Raman, K.B. Narayanan and N. Sakthivel. 2008. Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. B.M.C. Microbiol. p., 8-230.
- Natesan, R. and S. Shanmugasundaram. 1989. Extracellular phosphate solubilization by cyanobacterium anabaena ARM310. J. Biosci., 14: 203-208.
- Nautiyal, C.S. 1999. An efficient microbiological growth medium for screening phosphatesolubilizing microorganisms. FEMS Microbiol. Lett., 170: 265-270.
- Nishanth, D., Biswas, D.R., 2008. Kinetics of phosphorus and potassium release from rock phosphate and waste mica enriched compost and their effect on yield and nutrient uptake by wheat (Triticum aestivum). Bio Resource Technology., 99: 3342–3353.
- Owino, W.O., Y. Manabe., F.M. Mathooko., Y. Kubo and A. Inaba. 2006. Regulatory mechanisms of ethylene biosynthesis in response to various stimuli during maturation and ripening in fig fruit (Ficuscarica L.). Plant Physiol. Biochem, . 44: 335-342.
- Ozanne, P.G. 1980. Phosphate nutrition of plants: A general treatise. In: Khasawneh F.E., E.C. Sample and E.J. Kamprath (eds.). The Role of Phosphorus in Agriculture. Soil Sci. Soc., Am. Madison WI.
- Patten, C.L., B.R. Glick. 1996. Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol. 42: 207-220
- Persello-Cartieaux, F., L. Nussaume., and C. Robaglia 2003. "Tales from the Underground: Molecular Plantrhizobacteria Interactions." Plant, Cell and Environment., 26: 189- 99. Print.
- Penrose, D.M. and B.R. Glick. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth promoting rhizobacteria. Physiol. Plant., 118: 10-15.
- Puente, M.E., Y. Bashan., C.Y. Li and V.K. Lebsky. 2004. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. Plant Biol., 6: 629-642.

- Rajan, S.S.S. and B.C. Watkinson., 1993. Use of partially acidulated phosphate rocks as phosphate fertilisers. Fert. Res., 35: 47-59.
- Rajan, S.S.S. J.H. Watkinson., A.G. Sinclair 1996. Phosphate rocks for direct application to soils. Adv. Agron., 57: 77-159
- Rao, N.S. and Y.R. Dommergues. 1998. Microbial interactions in agriculture and forestry, Sci. Publ. Inc, U.S.A. 278 p.
- Rao, A.V. 2000. Soil biotechnological approaches for sustainable agricultural production in Indian arid zone, Microbiotech. 41st Ann. Conf. Assoc. Microbiologists of India. pp.
- Raghothama, K.G. and A. S. Karthikeyan. 2005. Phosphorus acquisition. Plant Soil., 274: 37-49.
- Rebi, A., javed, K., Kiran, S., Salman, Ghazafar, S., Ruby H. (2020) Effect of Applied Sulphur on Elemental Sulphur Contents in Maize. Int. J. of Food Sci. and Agric, 4(1), 1-5.
- Reyes, I., R. Baziramakenga., L. Bernier and H. Antoun. 2001. Solubilization of Phosphate rocks and minerals by an acid type strain and two UV-induced mutants of Penicillium rugulosum. Soil Biol. Biochem., 33:1741-1747.
- Richardson, A.E., R.J. Simpson. 2011 Soil microorganisms mediating phosphorus availability. Plant Physiol., 156: 989–996.
- Russell, E.W. 1973. The source of plant nutrients in the soil: Phosphate. In: Russell, E.W. (ed.). Soil Conditions and Plant Growth. Longman. London. 555-603. M.K. Saghir., A. Zaidi and P.A. Wani. 2007.
  Role of phosphate solubilizing microorganisms in sustainable agriculture - A Review. Agron.r Sust. Develop., 27: 29-43.
- Salamone, I.E.G. 2000. Direct beneficial effects of cytokinin producing rhizobacteria on plant growth. Ph.D. Thesis, University of Saskatchewan, Saskatoon, SK, Canada.
- Sander, L.J. 1986. Phosphorus for international agriculture. P. 87-90. In: Phosporus for Agriculture. A situation Analysis. Potash and phosphate Inst. Atlanta, GA.
- Sharpley, A.N., C.A. Jones., C. Gray and C.V. Cole. 1984. A simplified soil and plantphosphorus model: II. Prediction of labile, organic and sorbed phosphorus. Soil Sci.Soc. Am. J., 48: 805-809.
- Sharpley, A.N., Chapra, S.C., Wedepohl, R., J.T. Sims., T.C. Daniel., K.R. Reddy. 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual., 23: 437-451.
- Singh, H., N. Narda and J. Chawla. 2004. Efficacy of phosphorus through trickle fertigation of potato (Solanum tuberosum). Indian J. of Agric. Sci., 74: 476-478.
- Skrary, F.A. and D.C. Cameron. 1998. Purification and characterization of a Bacillus licheniformis phosphatase specific for D-alpha-glycerphosphate. Arch. Biochem. Biophys., 349:27-35.
- Smalberger, S.A., U. Singh., S.H. Chien., J. Henao., and P.W. Wilkens. 2006. Agron. J., 98:471-483.
- Sundra, B., V.Natarajan and K. Hari. 2002. Influence of phosphate solubilizing bacteriaon the changes in soil available phosphorus and sugarcane and sugar yields. Field Crops Res., 77: 43-49.

- Thaller, M.C., F. Berlutti., S. Schippa., P. Iori., C. Passariello and G.M. Rossolini. 1995b. Heterogeneous patterns of acid phosphatases containing low-molecular-mass Polipeptides in members of the family Enterobacteriaceae. Int. J. Syst. Bacteriol., 4:255-61.
- Timmusk, S., B. Nicander., U. Granhall and E. Tillberg. 1999. Cytokinin production by Paenibacillus polymyxa. Soil Biol.Biochem., 31: 1847-1852.
- Tunesi, S., Poggi, V., Gassa, C. (1999) Phosphate adsorption and precipitation in calcareous soils: the role of calcium ions in solution and carbonate minerals. Nutr. Cycling Agroecosyst., 53: 219–227.
- Vassilev, N., M. Vassileva., R. Azcon., 1997. Solubilization of rock phosphate by immobilized Aspergillus niger. Bio .Resource Technology., 59: 1-4.
- Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil., 255:571-586.
- Witt, C., and J.M.C.A. Pasuquin 2007. Maize in Asia and global demand II. E-Int. Fert. Corresp., 14: 5-6.
- Zahir, Z.A., M. Arshad and W.T. Frankenberger. 2003. Plant growth promoting rhizobacteria: Applications and perspectives in agriculture. Adv. Agron., 81: 97-168.
- Zaidi, A., M. S. Khan and M. Amil. 2003. Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (Cicer arietinum L.). Eur. J. Agron., 19:15-21.
- Zapata, F. and H. Axmann. 1995. 32P isotopic techniques for evaluating the agronomic effectiveness of rock phosphate materials. Fert. Res., 41: 189-195.