

# Phosphate Solubilizing Bacteria (PSB) – A Novel PGPR for Sustainable Agriculture

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**ABSTRACT:** The soil microorganisms are involving in the solubilization of fixed form or insoluble phosphorus includes bacteria, fungi, actinomycetes and arbuscular mycorrhizal fungi. Among them, Phosphate solubilizing Bacteria (PSB) plays an important role in making the phosphorus available to plants by mineralizing the organic phosphorus in the soil. PSBs have been isolated from a number of different soils in India and used as the potential biofertilizer. Their role in increasing the soil nutrient value is of almost importance and their application to crop fields has resulted in an increased growth, development and yield of several crop plants. The mechanism of P solubilization by PSB is the secretion of organic acids and phosphatase enzyme and these acid and enzyme able to solubilize the insoluble P and make it available to plants in the available form of P. In addition to P solubilization, PSB are capable of producing growth hormones such as auxins, cytokinins and gibberellin, vitamins, antibiotics, siderophores *etc.* Due to this property they enhance plant growth and suppress the soil borne pathogens and also improve physico-chemical properties of the soil and sustain soil fertility.

**KEYWORDS:** Phosphorus, PSB, solubilization, mechanism, crop response

## I. INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are group of bacteria that actively colonize plant roots and increase plant growth and yield by a wide variety of mechanisms and also offer an attractive way to replace chemical fertilizers, pesticides and supplements (Ashrafuzzaman *et al.*, 2009). PGPR influenced the plant growth by direct or indirect modes. Direct modes are the production of growth stimulators, improvement in plant nutrient status, release of phosphates and micronutrients from insoluble sources, lowering of the ethylene level in plant and induction of systemic resistance. Indirect modes of action are production of antibiotics, production of biocontrol agents and degradation of xenobiotics. PGPR are not only associated with the root to exert beneficial effects on plant development but also have positive effects on controlling phytopathogenic microorganisms (Son *et al.*, 2014; Pandey and Putatunda, 2018.).

The utilization of PGPR is now gradually increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides and supplements (Mandal *et al.*, 2017). Vejan *et al.* (2016) stated that the use of PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism. The mechanisms of PGPR include regulating hormonal and nutritional balance, inducing resistance against plant pathogens and solubilizing nutrients for easy uptake by plants. In addition, PGPR showed synergistic and antagonistic interactions with microorganisms within the rhizosphere and beyond in bulk soil, which indirectly boosts plant growth rate. The mechanism of PGPR is as a biofertilizer for agricultural sustainability.

The important PGPR are *Bacillus*, *Pseudomonas*, *Enterobacter*, *Azospirillum*, *Rhizobium*, *Erwinia*, *Serratia*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Acinetobacter* and *Flavobacterium* (Rodriguez and Fraga, 1999) and some of them have been commercialized, including the species *Pseudomonas*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Azobacter*, *Variovorax*, *Azospirillum* and *Serratia*. However, the utilization of PGPR in the agriculture industry represents only a small fraction of agricultural practice worldwide (Bashan *et al.*, 2014). The successful utilization of PGPR was dependent on its survival in soil, the compatibility with the crop on which it was inoculated, the interaction ability with indigenous microflora in soil and environmental factors (Martinez-Viveros *et al.*, 2010).

# International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

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Vol. 7, Issue 4, April 2018

## II. RELATED WORK

PSB includes bacteria, fungi, actinomycetes and even algae. Bacteria are predominant amongst them and proved more effective in phosphate solubilization than fungi. In addition to *Pseudomonas* and *Bacillus*, other bacteria reported as P-solubilizers these are *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Phyllobacterium* etc. (Wani *et al.*, 2005). Several soil bacteria, particularly those belonging to the genera *Pseudomonas* and *Bacillus* possess the ability to convert the insoluble phosphate into soluble form by secreting organic acids resulting in improved phosphate availability to the plants are called PSB (Fankem *et al.*, 2006). PSB play a major role in correcting phosphorus deficiency of crop plants. These microorganisms may also release soluble inorganic phosphates into soil through decomposition of phosphate rich organic compounds. The recent trend of nutrient supply approach for improving agricultural production has given greater emphasis on the use of biofertilizers along with chemical fertilizers (Dave and Patel, 1999). Phosphate solubilizing microbes dissolves the phosphorus content in soil through the formation of organic acids and reduces the pH of the rhizosphere. This PSM based biofertilizers improve the quality of fruits, vegetables and grain crops and increase their resistance to diseases and adverse condition (Anbuselvi *et al.*, 2015).

Phosphate solubilizing bacteria play an important role in converting low grade insoluble inorganic phosphate sources like rock phosphate, bone meal, basic slag and the chemically fixed soil phosphorus into available form. Therefore, the use of phosphate solubilizing microbes in agricultural practice would not only offset the high cost of manufacturing phosphatic fertilizers but would also mobilize insoluble phosphorus in the fertilizers and soils to which they are applied. The mechanism of solubilization of insoluble phosphate is ability to secrete organic acids and phosphatase enzyme (Vessey, 2003; Mahantesh *et al.*, 2015).

Apart from the solubilization of fixed form of P, PSB can also able to produce the plant growth promoting hormones like auxins, gibberellins and cytokinins. These hormones induce plant growth as well as dry matter production. Inoculation with PSB resulted in better growth and higher dry matter production in maize and is mainly attributed to solubilization of phosphate and the production of plant growth hormones. The PSBs are able to synthesize phytohormones like indole acetic acid, gibberellic acid (Ramkumar and Kannapiran, 2011) and siderophore (Babna *et al.*, 2013). PSBs are also enhances plant growth by increasing the efficiency of biological nitrogen fixation of other trace elements such as iron, zinc, etc. (Ponmurugan and Gopi, 2006). Phytohormones play an important role as growth regulator such as auxins and cytokinin are responsible for division, extension and differentiation of plant cells and tissues (Tsavkelova *et al.*, 2006).

Phosphate solubilizing bacteria of *Pseudomonas* and *Bacillus* species are producing phytohormones *i.e.* auxins, inhibition of deleterious pathogens or nutrient mobilization and ammonification. The plant growth benefits due to the addition of PSB include increases in germination rate, root growth, yield, leaf area, chlorophyll content, tolerance to drought, shoot and root weight. Further, the inoculation of PSB as biofertilizers enhanced the P accumulation and biomass production of plants (Abbasi *et al.*, 2015). The action of PSB was not only due to the release of plant available phosphorus but also the production of biologically active substances like indole acetic acid, gibberellins; cytokinins production was also correlated with P solubilization. The favourable effect of the inoculants on plant growth and nutrient uptake was due to the production of growth promoting substances by PSB (Kundu *et al.*, 2002). The available phosphorus content of the soil, nodulation, root and shoot biomass, straw and grain yield were increased due to PSB inoculation as compared to the inoculated controls. PSB could increase growth and yield in several crops including walnut (Xuan *et al.*, 2011), apple (Aslantas *et al.*, 2007), maize (Hameeda *et al.*, 2008), soybean (Fernandez *et al.*, 2007), chickpea (Akhtar and Siddiqui, 2009) and peanut (Taurian *et al.*, 2010).

The phosphate solubilizing bacteria are life forms that can help in improving phosphate uptake of plantain on different ways (Rajasekaran *et al.*, 2012). PSBs have the ability to promote plant growth. These bacteria can enhance plant growth and increase plant P content. Tobacco plants with the lowest dry weights were grown in unfertilized soil This was likely due to P deficiency in the soil used to grow these seedlings. PSB inoculation, increased biomass yield relative to the treatment, indicating that inoculation of PSB strains enhanced biomass, presumably by solubilizing soil P (Basak and Biswas, 2010).

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Vol. 7, Issue 4, April 2018

## III. PHOSPHATE SOLUBILIZING BACTERIA (PSB)

Phosphorus is one of the major plant nutrients required in optimum amount for plant growth. Some heterotrophic bacteria and fungi are known to have the ability to solubilize inorganic phosphate from insoluble sources and making available to plants. These microorganisms are known as phosphate solubilizers. The most efficient phosphate solubilizing bacteria include *Bacillus* and *Pseudomonas* and that of fungi include species of *Aspergillus* and *Penicillium* (Baby *et al.*, 2001). Microorganisms that involved in the solubilization of insoluble P include eubacteria, fungi, cyanobacteria, actinomycetes and arbuscular mycorrhizal fungi (Khan *et al.*, 2007). Phosphate solubilizing microorganisms include fungi (*Penicillium* and *Aspergillus*), bacteria (*Pseudomonas*, *Bacillus*, *Rhizobium*, *Agrobacterium*, *Micrococcus*, *Enterobacter* and *Erwinia*) (Gulati *et al.*, 2007). Phosphate solubilizing microorganisms brings about mobilization of insoluble phosphates in the soil and increase plant growth under conditions of poor phosphorus availability (Tripura *et al.*, 2007). Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi were the most powerful P solubilizers. *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata* and *Enterobacter* could be referred as the most important strains (Kucey *et al.*, 1989).

Phosphate solubilizing bacteria have significant role in making phosphorus available to plants by bringing about favourable changes in soil reaction in the soil microenvironment leading to solubilization of inorganic phosphate sources. Some microorganisms associated with different plant rhizosphere are able to solubilize inorganic insoluble P salts (Yadav and Tarafdar, 2011). PSB play important role by enhancing its availability to plants through release from inorganic and organic soil P by solubilization and mineralization. The ability of microorganisms to convert insoluble phosphorus to a soluble form, like orthophosphate, was an important trait in a plant growth promoting bacteria for increasing plant yields. The inoculation of P-solubilizing microorganisms was an efficient technique because it can increase P availability in the soil (Sanjotha and Manawadi, 2016). PSB hold most important position in P-solubilizing soil microbes due to their multifunctional capabilities. Along with enhancing phosphorus availability for the plants, some PSB were also responsible for production of siderophore (Wani *et al.*, 2008), indole acetic acid and gibberellin (Souchie *et al.*, 2007), antibiotics (Taurian *et al.*, 2010) and secondary metabolites (Wani *et al.* 2007).

## IV. MECHANISM OF PHOSPHATE SOLUBILIZATION BY PSB

The mechanism of phosphate solubilization by PSB may involve the release of low molecular weight of organic acids, which chelate phosphate bound cations to convert P into soluble forms (Castagno *et al.*, 2011); mechanism of phosphate solubilizing includes production of organic acids (Hu *et al.*, 2009) and production of acid phosphatase (Turner and Haygarth, 2005). Phosphorus solubilizing activity was determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe *et al.*, 1998). Phosphorus solubilization was carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation mediated mechanisms (Whitelaw, 2000). Inorganic P was solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, and Ca) and decrease the pH in basic soils.

The phosphate solubilization by the *Pseudomonas fluorescens*, *Bacillus megaterium* and *Azospirillum* spp. was accompanied with pH reduction of the culture medium. Maximum pH reduction was 2.8, 1.2 and 0.5 units for *Pseudomonas fluorescens*, *Bacillus megaterium* and *Azospirillum lipoferum* strain, when compared to control pH of 6.8 (El-Komy, 2005). The lowering in pH of the medium suggested that the secretion of organic acids by the P-solubilizing microorganisms (Maliha *et al.* 2004) via direct oxidation pathway that occurred on the outer face of the cytoplasmic membrane (Zaidi *et al.* 2009). When P was 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.

PSB strains were able to produce more organic acids or having an effective phosphatase enzyme by which it solubilize maximum amount of phosphate. Organic acids were found to be responsible for tricalcium phosphate and rock phosphate solubilization (Kumari *et al.*, 2008). Addition of EDTA was found to increase solubilization of

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Vol. 7, Issue 4, April 2018

tricalcium phosphate. Organic acids were also found to mediate the release of phosphorus bound to iron oxides (Johnson and Loeppert, 2006). P-solubilizing microbes excrete inorganic acids, organic acids and phosphatase to dissolve phosphorus (Zhong *et al.*, 2014). However, the mechanisms of P-solubilization not only differ among microorganisms but also dependent on the applied P sources (de Oliveira Mendes *et al.*, 2014).

Roca *et al.* (2013) found that the high alkaline phosphatase enzyme produced by PSB and were responsible for organic solubilization of insoluble phosphate. However, phosphatase was not directly act on inorganic phosphate solubilization, though phosphatase activity may participate in lowering the pH of the culture medium by the dephosphorylating action and the production of acids (Achal *et al.*, 2007). Phosphate solubilization of most organic phosphorous compounds is carried out by means of phosphatase enzymes. The presence of a significant amount of phosphatase activity in soil has been reported (Lynch *et al.*, 1991). Important levels of microbial phosphatase activity have been detected in different types of soils (Kurchaski *et al.*, 1996). In fact, the major source of phosphatase activity in soil is considered to be of microbial origin (Garcia *et al.*, 1992). In particular, phosphatase activity is substantially increased in the rhizosphere (Assefa and Fenta, 2017).

## V. PREPARATION OF BIOINOCULANTS WITH PSB

Bioinoculants are usually prepared as carrier based inoculants containing effective microorganisms. Assimilation of microorganisms in carrier materials enables easy handling, long term storage and high effectiveness of biofertilizers. Since the beginning of bioinoculants use at large scale, several carrier materials have been tried like Farm Yard Manure (FYM), compost, peat soil, coal, charcoal, cellulose powder, lignite, talc, bagasse, sedge peat, press mud, teak leaf meal, coconut shell powder *etc.* Although, fly ash was basic in nature and it has good mineral content. Use of fly ash in soil as bioformulation was useful because it increases pH of soil, converts the nutrients in available form, supplies nutrients to soil and it solves disposal problem of fly ash (Dwivedi and Chauhan, 2007). A cost effective carrier materials which were non polluting, biodegradable, non-toxic, capable of maintaining high viable count and long shelf life amendable to nutrient supplement and high water holding capacity (Gomare *et al.*, 2013). Quality of the bioinoculants was one of the most important factors deciding their field performance (Baby, 2004).

## VI. CROP RESPONSE OF PSB

Plants were unable to uptake insoluble phosphorus but acquire phosphorus only in soluble form as a phosphate anion. In recent years, researches showed that certain microbial strains have the ability to utilize beneficiary elements present in the soil, initiating plant growth. The groups of microorganisms which bear the potential to hydrolyze organic and inorganic phosphorus from insoluble compounds into simpler soluble forms are referred to as Phosphate Solubilizing Microorganisms (PSM). Therefore PSMs can be used as biofertilizer amendments and inoculums of the microbial strains upon implementation can help in achieving crop improvement (Pirlak and Kose, 2009; Cao *et al.*, 2018).

PSB as inoculants increased the P uptake by the plants and crop yield. Strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* were among the most powerful phosphate solubilizers (Hayat *et al.*, 2010). The substantial increase in plant growth was due to the single, dual or three member associations of rhizospheric microorganisms (Zaidi and Khan, 2007). Specific strains of the *Pseudomonas fluorescens* and *Pseudomonas putida* were rapidly colonized the plant roots of several crops and cause statistically significant yield increase. Under phosphorus deficient conditions in improved uptake of the available phosphates or rendering unavailable phosphorus sources accessible to the plant. It has been found that *Pseudomonas* spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (Son *et al.*, 2006).

Turan *et al.* (2007) stated that the application of PSB significantly increased soluble P and plant P uptake in tomato plant seedling. The amount of P uptake in plant tissue differed significantly between bacterial strains. A greater increase was noticed in plant phosphorous uptake in treatments with PSB application than without PSB in all of treatments. Tantawy and Mohamed (2009) also observed the effect of inoculation with phosphate solubilizing bacteria on tomato rhizosphere, colonization process plant growth and yield. The inoculation with *Pseudomonas* spp. had a positive significant effect on tomato leaves phosphorous content. The PSB as inoculants simultaneously increased the P uptake by the plant and crop yield. The PSB exhibiting multiple plant growth promoting traits on soil-plant system was

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Vol. 7, Issue 4, April 2018

needed to uncover their efficiency as effective bio inoculants. The inoculation of PSB and plant growth promoting rhizobacteria together could reduce 50% of P fertilizer application without any significant decrease of crop yield (Sharma *et al.*, 2013). PSBs were autochthonous and their population was not sufficient to compete with other soil bacteria. Therefore, application of these PSB as bioinoculants in agricultural practice was expected to improve crop yield and productivity.

PSB improved the plant growth, yield and phosphorus content of several crops and used as bioinoculant to enhance the sustainable production. All strains showed a positive effect on plant growth. A significant increment in plant height, shoot dry weight was determined in plants treated with *Pseudomonas tolaasii*, while *Pseudomonas koreensis* has remarkably increased P content compared to the uninoculated control. *Pseudomonas* strain was selected and evaluated under field conditions in combination with triple superphosphate as P fertilizer. The presence of *Pseudomonas* strain stimulated seedling emergence, shoot length, grain yield, grain weight, total dry biomass and P content of maize plants. In general, *P. tolaasii*, inoculation was more efficient as bioinoculant without P fertilizer (Viruel *et al.*, 2014).

The application of PSB either individually or combined, effected on the growth and biomass production of several crops (Hariprasad and Niranjana, 2009). Biofertilizer produced the plant growth regulating substances, which promotes root growth found the significant effect on number of primary flowers number of secondary flowers, total number of flowers, number of primary fruit, number of secondary fruit and total number of fruit by the application of PSB in strawberry (Zargar *et al.*, 2008). Sole application of bacteria increased the biological yield, while the application of the same bacteria along with mycorrhiza achieved the maximum grain weight (Mehrvarz *et al.*, 2008). Single and dual inoculation along with P fertilizer was 30-40 % better than P fertilizer alone for improving grain yield of wheat and dual inoculation without P fertilizer improved grain yield up to 20% against sole P fertilization. Plant growth promotion and increased phosphorous availability due to inoculation of PSMs have been assessed in several studies under green house as well as field conditions (Zaidi *et al.*, 2003).

Several field experiments examined the influence of the bio-inoculation by PSB and *Rhizobium* by evaluating different symbiotic traits such as nodule leghemoglobin. Indeed, it was reported that, through an evaluation of *Rhizobium* associated with PSB inoculants of chickpea, the yield was higher in terms of nodule number, nodule fresh weight, nodule dry weight, shoot dry weight and leghemoglobin content. Moreover, this bio-inoculation showed a significant enhancement in terms of grain and straw yield (Tagore *et al.*, 2013). A large number of researches demonstrated that PSB strains could improve plant growth such as *Brassica napus* L. (Freitas *et al.*, 1997), *Zea mays* L. (Nadeem *et al.*, 2007), *Brassica juncea* (Kumar *et al.*, 2009), *Stevia rebaudiana* (Mamta *et al.*, 2010), sugarcane (Beneduzi *et al.*, 2013) and cotton (Wu *et al.*, 2014). PSB treatment increased the plant height, stem thickness, root and shoot dry weight of *V. vinifera*. It was probable that PSB enhanced the amount of soluble P in soils and consequently increasing P uptake of grapevine. A significant difference in the root, shoot length and shoot dry weight of tomato plants was observed at 60 days of plant growth due to various inoculation treatments. The treatment receiving inoculation of PSB5 recorded maximum root length and was significantly superior over all other inoculation treatments followed by PSB7. All treatments showed significant increase in root growth over absolute control (Assefa and Fenta, 2017).

Application of compost enriched with PSB and rock phosphate, significantly increased the grain yield of rice and black gram in an experiment conducted by Manna *et al.* (2001). Singh *et al.* (2009) studied the utilization of rock phosphate through phosphorus compost in rice-wheat system in a field experiment and found increase in the relative yield of rice and wheat by 110 and 104 % respectively. The stimulative effect of PSB on growth and consequently on dry weight may be due to the activity of P solubilization further mineral availability uptake and plant growth (Han *et al.*, 2006). The evidenced increase in plant height and fresh weight might be attributed to better to NO<sub>3</sub>, nutrients availability in soil and uptake in treated plants.

## VII. CONCLUSION

With the growing awareness about and concerns for deteriorating soil health and increasing pressure for making plant nutrients available at reasonable cost, efforts are being made to exploit microbial resources (PGPR) for crop nutrients. In this circumstance, PSB biofertilizers are being promoted as important component of integrated nutrient management approach especially for soil P content. With the emergence of organic farming, the contribution of

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Vol. 7, Issue 4, April 2018

biofertilizers has further gained importance. Research fraternity and Industrialist have to share the farm duties to overcome the constraints and offer suitable, fail-proof biofertilizer input technologies to farmers.

## VIII. ACKNOWLEDGEMENT

The authors are thankful to the Management and the Principal of Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu for providing laboratory facilities and financial assistance to carry out this research work.

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