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Review Article

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Utilization of phosphate solubilizing bacteria (PSB) for sustainable agriculture

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ABSTRACT

After Nitrogen Phosphorus is the second most required macronutrient for plants. It is required from molecular level to physical development of plants. Most of the soils contain high levels of Phosphorus. However P forms many insoluble complexes with Calcium, Iron, and Aluminium. It makes the nutrient a paradox. It is reported to be critical factor of many crop production systems due to limited plant available P forms in soil. 80% Phosphorus from soil remains unutilized. Phosphate solubilizing bacteria provide an eco-friendly alternative to convert insoluble phosphate into soluble forms. Species of PSB like Bacillus, Rhizobium and Pseudomonas have ability to release metabolites such as organic acids to carry out mineral phosphate solubilization. The present review is focused on an urgent need of shifting towards a more sustainable agriculture by using PSB.

Keywords: Rhizosphere, Phosphorus, Phosphate Solubilizing Bacteria.

INTRODUCTION

After Nitrogen Phosphorus (P) is the second most essential macronutrient, for growth and development of plants as it is involved in important metabolic pathways like photosynthesis, respiration, biological oxidation, nutrient uptake, cell division and cell building (Pathak *et al.*, 2017; Gupta *et al.*, 2012). Soils contain high levels of P, but, 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 (Muhammad and Maran, 2012; Kannapiram and Sri Ramkumar, 2011). Unlike the case of Nitrogen there is no large atmospheric source of Phosphorus that can be made Biologically available to plants (Karpagam and Nagalaxmi 2014). Conventional farming system relies on heavy application of chemical phosphorus fertilizers to maintain optimum level of phosphorus in agricultural soils (Ahmed and Kiber, 2014). However, major portions (around 75% in some soils) of the soluble phosphorus are rapidly immobilized in soil which makes it unavailable for plants (Richardson and Simpson, 2011). Also the repeated use of chemical fertilizers deteriorates soil quality (Gyaneshwar et al, 2002).

The soil surrounding the germinating seed is known as spermosphere (Barillot *et al.*, 2013). This ecological niche is the first habitat used by any developed microorganism by seed inoculation and support microbial activity (Ranjan *et al.*, 2013). This in turn influences plant growth. As the plant grows older the growing roots are surrounded by strongly adhering soil particles and this soil-root interaction zone is the rhizosphere (Kumar *et al.*, 2018; Antoun, 2012). The rhizosphere are said to be the areas of very high biological diversity teaming with many different organisms, as the plant roots provide food, shelter and energy (Barillot *et al.*, 2013). These active microbial population can either exert beneficial or detrimental effects or they can be neutral (Sunder-Rao and Sinha, 1963). The success in the use of beneficial microorganisms requires an excellent understanding between the different components of the complex plant-soil-microorganisms (Gyaneshwar *et al.*, 2002)

Natural solubilization of mineral phosphates is an important mechanism exhibited by different rhizospheric microorganisms, known as phosphate solubilizing microorganisms (PSM) (Selvi *et al.*, 2017). Bacteria are the predominant microorganisms that solubilize mineral phosphate in nature, as compared to other microorganisms (Paul and Sinha, 2017; Debojyoti *et al.*, 2015). Phosphate solubilizing bacteria (PSB) play an important role in biogeochemical phosphorus cycling in both terrestrial and aquatic environments (Das *et al.*, 2007). Application of phosphate solubilizing bacteria increases soil fertility due to their ability to convert insoluble P to soluble P by releasing organic acids, chelation and ion exchange (Muhamad and Maran, 2012; Selvi *et al.*, 2017; Tarafdar and Classon, 1988). The present review emphasises the role of Phosphorus in plant nutrition, PSB, mechanism of phosphate solubilization and utilization of PSB for sustainable agriculture.

ROLE OF PHOSPHORUS IN PLANT NUTRITION

Phosphorus (P) is a most important growth limiting nutrient for plants (Richardson and Simpson, 2011). Unlike the case of Nitrogen, there is no large distinctive source that can be naturally managed for crops accessibility (Sharma *et al.*, 2013). P, being important constituent element of nucleic acids, enzymes, coenzymes, nucleotides and phospholipids, is involved in the transformation of energy, transfer of hereditary characters and cell organization in plants (Rodriguez and Fraga, 1999; Goldstein, 1986). Nearly all phases of plant cycle including root growth, stalk and stem strength, photosynthesis and respiration, flowering and anthesis, seed formation, crop maturity and production N-fixation in legumes, crop quality and resistant to plant diseases are the attributes associated with P nutrition(Balemi and Negisho, 2012; Satyaprakash *et al.*, 2017). Its deficiency causes stunted growth and severe yield losses. Its concentration in soil solution is very low, because soluble forms of P are fixed by soil solid phase, making less than 0.01% of total P available to plants (Trolov *et al.*, 2003). Phosphorous is therefore, one of the least mobile nutrients in soil (Balemi and Negisho, 2012; Gyaneshwar, 2012). Worldwide soils are supplemented with inorganic P as chemical fertilizers to support crop production, but repeated use of fertilizers deteriorates soil quality (Vessey, 2003). In India about 98% of soils including fertile ones are deficient in P, as the concentration of free P (available form for plants) is not more than 10μm even at pH 6.5 (Debojyoti *et al.*, 2015). Thus, most of the Indian soils are poor and marginal in phosphorus and require adequate P-fertilization to sustain high productivity and profitability (Debojyoti *et al.*, 2015). Phosphorus mainly occurs in three forms (i) soluble inorganic-P which occurs in soil solution (ii) insoluble inorganic P occurring as primary orthophosphate (H_2PO_4) and (iii) secondary orthophosphate (HPO_4^{2-}) (Goldstein, 1986). The form of phosphate taken up by the plant from soil solution is phosphate anions mainly H_2PO^{+} and HPO_4^{2-} (Richardson, 2001). Many agricultural soils surround huge deposition of total P, generally from 200 to 5000 mg P kg^{-1} of soil by mean value of 600 mg P kg^{-1} of soil and its accumulation based on frequent use of inorganic fertilizers and sludge from the treated wastewaters (Rodriquez and Fraga, 1999). Much of the P which is present in soil and provided to the crops through inorganic fertilizers become unavailable through precipitation by reacting with Fe^{3} and Al^{3+} in acidic and with Ca^{2+} in calcareous soils, to form Aluminium Phosphate ($AlPO_4$) and Ferrous Phosphate ($FePO_4$) which are sparingly soluble precipitates (Sharpley, 1985) and ultimately resulting in the low concentration of P in soils making it unavailable to plants (Pikovskaya, 1948). Therefore, crop plants can ... only a fraction of applied P and this make them poor in performance (Abbasi *et al.*, 2015). The concentration of P in soil solution is very low, varying from 0.001mg L^{-1} in very poor soils to 1mg L^{-1} in heavily fertilized soils (Seema *et al.*, 2013).

PHOSPHATE SOLUBILIZING BACTERIA (PSB)

The fact that certain soil microbes are capable of dissolving relatively insoluble phosphatic compounds has opened the possibility for inducing microbial solubilization of phosphates in soil (Ahmed and Kiber, 2014). Phosphate solubilizing microorganisms are the one which solubilize the insoluble form of phosphate in the soil to make it available for the plant growth (Eivazi and Tabatabai, 1977). Phosphate solubilizing microorganisms include several Bacteria, Fungi, Actinomycetes, Yeast and Cyanobacteria (Saxena *et al.*, 2016; Yazdani *et al.*, 2009; Chen *et al.*, 2006; Khan and Zaidi, 2006). But bacteria are the predominant microorganisms that solubilize mineral phosphate in nature, as compared to other microorganisms (Paul and Sinha, 2017; Zaidi and Khan, 2007).

PSB play a vital role in mobilizing Phosphate for the use of plants from the native soil Phosphorous pools as well as rock phosphates (Sharma *et al.*, 2013; Trolov *et al.*, 2003). Several phosphate solubilizing bacteria can utilize insoluble phosphate sources such as tri-calcium phosphate (TCP), hydroxyapatite, flourapatite, ferric, Aluminium and magnesium phosphate, bone meal and rock phosphates and convert them into soluble forms (Muhamad and Maran, 2012; Gaur *et al.*, 1973; Eivazi and Tabatabai, 1977). The important genera of P-solubilizing bacteria include *Achromobacter*, *Aerobacter*, *Alcaligenes*, *Azotobacter*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Serratia* and *Xanthomonas* (Linu *et al.*, 2009; Afzal and Bano, 2008; El-Tarabily *et al.*, 2008; Premono *et al.*, 1996), *Azospirillum* (Mehmet *et al.*, 2005). Among these, bacteria belonging to genera *Pseudomonas*, *Bacillus* and *Rhizobium* and fungi belonging to genera *Penicillium* and *Aspergillus* possess the greater ability to solubilize the insoluble phosphates (Singh and Siddiqui, 2015, Chhabot *et al.*, 1996). The efficiency of these strains depends on various factors such as temperature, soil, pH, type of insoluble phosphate etc. (Ahmed and Kiber 2014, Vessey, 2003; Eivazi and Tabatabai, 1977). The phosphate solubilizing bacteria can be isolated from different sources such as soil (Barillot *et al.*, 2013 Chen *et al.*, 2006; Mehta *et al.*, 1954), rhizosphere (Dalvi *et al.*, 2019; Gyaneshwar *et al.*, 2002), rootnodules (Vikram and Hamzehzarghani, 2008) and compost (Zalate and Padmani, 2009; Panwar *et al.*, 2002)). Inoculation with phosphate solubilizing organisms in multilocational trials conducted with wheat, paddy, chickpea, maize, greengram, lentils and potato have shown increased yields (Trolov *et al.*, 2003).

MECHANISM OF PHOSPHATE SOLUBILIZATION

Bacteria are the predominant microorganisms that solubilize mineral phosphate in nature, as compared to other microorganisms (Paul and Sinha, 2017). Phosphate solubilizing bacteria (PSB) play an important role in biogeochemical phosphorus cycling in both terrestrial and aquatic environments (Das *et al.*, 2007). Application of phosphate solubilizing bacteria increases soil fertility due to their ability to convert insoluble P to soluble P by releasing organic acids, chelation and ion exchange (Omar, 1998; Narula *et al.*,2000.).

Phosphate solubilization takes place through various microbial processes/mechanisms including organic acid production and proton extrusion, the principal mechanism being the lowering of soil pH by microbial production of organic acids or the release of protons and mineralization by producing acid phosphatases (Tarafdar and Claasen, 1988), ultimately resulting in P availability in soil (Whitelaw, 2000). Soil phosphates mainly of phosphatic fertilizers under alkaline conditions are fixed in the form of insoluble phosphates. Many of the calcium phosphates, including rock phosphate ores (fluoroapatite, francolite), are insoluble in soil. Their solubility increases with a decrease of soil pH (Gyaneshwar *et al.*, 2002).

Phosphobacteria have been found to produce some organic acids such as monocarboxylic acid (acetic, formic), monocarboxylic hydroxy (lactic, glucenic, glycolic), monocarboxylic, ketoglucenic, decarboxylic (oxalic, succinic), dicarboxylic hydroxy (malic, maleic) and tricarboxylic hydroxy (citric) acids in order to solubilize inorganic phosphate compounds (Muhamad and Maran, 2012; Eivazi and Tabatabai, 1977). The type of organic acid produced and their amounts differ with different organisms (Karpagam and Nagalaxhmi, 2014). Among them, gluconic acid and 2-ketogluconic acid seems to be the most frequent agent of mineral phosphate solubilization (Deubel *et al.*, 2000; Song *et al.*, 2008). The organic acids are the products of the microbial metabolism, mostly by oxidative respiration or by fermentation of organic carbon sources like glucose (Trolove *et al.*, 2003).

Phosphate solubilization is the result of combined effect of pH decrease and organic acids production (Fankem *et al.*, 2006, Suranga and Kumar, 1993). PSB decrease the soil pH by producing organic acids (Sardina *et al.*, 1986). These organic acids compete with the P binding sites in the soil (Nahas, 1996). Through Organic acid production PSB mobilizes P from sparingly soluble phosphates and convert into soluble forms mainly by the chelation mediated mechanism (Whitelaw, 2000). Acidification of the surroundings of microbial cell releases proton through the production of organic acids (Villegas and Fortin, 2002). These metabolite organic acids as a result of anion exchange of P by an acid anion can either directly dissolve the mineral P or can chelate Al^{3+}, Fe^{3+}, Ca^{2+} ions associated with inorganic P (Gupta *et al.*, 1993). The carboxyl and hydroxyl groups chelate the cation bound to inorganic P and convert it into soluble forms (Sagoe, 1998). As the soil pH increases the divalent and trivalent forms of inorganic P, $HPO4^{-2}$ and $HPO4^{-3}$ occur in the soil (Suranga and Kumar, 1993). The organic acids released by PSB into the soil consequently decreases the soil p^{H} and acidifies the surrounding environment and lead to the release of P ions by H^{+} substitution for the cation bound to phosphate (Goldstein, 1986) making the P available to plants. PSB mineralize soil organic P by the production of acid phosphatases. Release of-organic anions, and production of Siderophores and acid phosphatase by microbes, hydrolyze the soil organic P or split P from organic residues resulting in P availability (Dodor and Tabatabai, 2003).

UTILIZATION OF PHOSPHATE SOLUBILIZING BACTERIA (PSB) FOR SUSTAINABLE AGRICULTURE

As a result of energy crisis, environmental hazards and depleting soil fertility, man needs to shift towards a more sustainable agriculture (Kumar *et al.*, 2018). Chemical fertilizers should be replaced with biofertilizers,the latter being eco-friendly, productive and accessible (Sheraz *et al.*, 2010). PSB play a crucial role in bio-geo phosphorus cycle as they affect the transformation of the soil phosphates (Sharpley, 1985). They increase nutrient uptake from soil, thus reducing the need for fertilizers and preventing accumulation of phosphates in soil. The concentration and number of PSB in rhizosphere can be inadequate for the complete supply of inorganic phosphates to the plants (Sunder-Rao and Sinha, 1963). Therefore inoculation of the plants with these phosphate solubilizers is essential for efficient phosphate solubilization (Karpagam and Nagalaxmi, 2014).

Several workers concluded that application of phosphate-solubilizing microorganisms increases soil fertility and results in growth promotion of crops (Omar 1998; Narula *et al.*, 2000). The positive effect of P solubilizers has been reported on food and fodder crops (Dalvi *et al.*, 2019; Santana *et al.*, 2016; Gupta et al, 2012). Phosphate solubilizing bacteria are being used as biofertilizers since 1940's (Cerretsen, 1948; Krasilinikov, 1957; Vssey, 2003). Inoculation of seeds with PSB is a promising technique which alleviates the deficiency of P (Qureshi *et al.*, 2012). Inoculation with PSB such as *Pseudomonas* (Husen *et al.*, 2009), *Bacillus* (Turan *et al.*, 2007; Mehejibin and Patel, 2007), *Rhizobium* (Afzal and Bano, 2008; Chabot *et al.*, 1996), *Micromonaspora* (El-Tarabily *et al.*, 2008), *Burkholderia* (Minaxi and Saxena, 2010), *Azotobacter* (Naseri and Mirzai, 2010), *Acinetobactor* (Gulati *et al.*, 2010), *Azospirillum* (Nesri and Mirzai, 2010; Mehmet *et al.*, 2005) and *Gluconacetobacter* (Linu *et al.*, 2009) has been reported in increasing solubilization of fixed P ensuring high crop yields (Rodriguez and Fraga, 1999).

Bacteria belonging to the genus *Baccilus*, *Pseudomonas*, and *Rhizobium* have proven to be powerful PSB (Saxena *et al.*, 2016; Noori and Saud, 2012, Turan *et al.*, 2007, Mhejibilin *et al.*, 2007). Savankumar and Samiyappan, 2006, studied ACC deaminase from *Pseudomonas flurorescens* mediated saline resistance in groundnut. Bhatia *et al.*, 2008, Showed beneficial effects of fluorescent Pseudomonads on growth promotion and suppression of charcoal rot in groundnut. Studies on *Pseudomonas* and *Trichoderma* proved phosphate solubilisation and antagonistic activities against *Fusarium oxysporum* and *Rhizoctonia solani* (Jay et al., 2014). *Rhodococcus* sp, *Pseudomonas* sp. and *Arthrobacter nicotinovorans* when inoculated with *Zea mays* and grown in P deficient soils amended with tricalcium phosphate enhanced the plant growth (Sofia and Paula, 2014). *Pseudomonas* sp., *Serratia marcescens* and *Bacillus cereus* were known to reduce the bacterial wilt caused by *Ralstonia solanacearum* in tomato (Sing and Siddiqui, 2015) and hence proved to be effective biocontrol agents (Henok and Kerstin, 2013). Bano and Mussarat, 2003, Naseby *et al.*, 2001 and Haas and Keel, 2003, also observed biocontrol properties of *Pseudomonas* strains.

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Several workers reported potency of PSB in solubilising Phosphorous and increasing yield in crops as Tomato (Pathak *et al.*, 2017; Sharon *et al.*, 2016; Lamsal, 2013 ; Poonia and Dhaka, 2012; Hariprasad and Niranjan, 2009), Maize (Mohamed *et al.*, 2017; Hussain *et al.*, 2013; Gholami *et al.*, 2009 ; Yazdani *et al.*, 2009; Nadeem *et al.*, 2010), Wheat (Sachdev *et al.*, 2009; Afzal and Bano, 2008; Babana and Antoun, 2006; Mehmet *et al.*, 2005; Khalid *et al.*, 2004; Nerula *et al.*, 2000), Soyabean (Husen *et al.*, 2009; El-Tarabily *et al.*, 2008), Moog bean (Walpola and Yoon, 2013; Jha *et al.*, 2012; Minaxi and Saxena, 2010; Vikram *et al.*, 2008), Cicer (Sharma *et al.*, 2015; Zaidi and Khan, 2007), Groundnut (Zalate and Padmani, 2009; Bhatia *et al.*, 2008; Panwar *et al.*, 2002), Cotton Qureshi *et al.*, (2012)., Sunflower (Zehara, 2010), Safflower (Zhang *et al.*, 2019), Sugarcane (Sundara *et al.*, 2002), Sorghum (Gopalkrishna and Humayun, 2011) , Chilli (Abbasi *et al.*, 2015) and Mash bean (Niazi *et al.*, 2015).

Some workers noticed additional features of PSB to facilitatate plant growth and development in the presence of various stresses. *Pseudomonas* sp. enhanced plant growth in salt stress (Nadeem *et al.*, 2010; Mohammad *et al.*, 1998). *Acinetobacter* (Gulati *et al.*, 2010), *Pseudomonas putida* (Pandey *et al.*, 2006) and *Pseudomonas corrugate* (Trivedi and Sa, 2008) are observed to be cold tolerant. *Pseudomonas* sp. (Sandhya *et al.*, 2010), *Arthrobacter* sp., *Bacillus* sp. (Banerjee *et al.*, 2010) showed tolerance towards drought stress. *Pseudomonas fluorescence Bacillus* and *Hallobacillus* showed tolerance towards salinity stress (Saravanakumar and Samiyappan, 2006).

The review concludes that phosphate solubilizing bacteria promise a better alternative to the chemical fertilizers, the latter being costly, deteriorating the agriculture soils and environmental concern. Phosphate solubilizing biofertilizers will prove eco-friendly and cost effective agro technology to improve crop production. More research in this field is needed to minimize the use of chemical fertilizers and make use of biofertilizers in large scale for sustainable agriculture.

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