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Phosphate Solubilizing Microbes: A Biological Solution for 'P' Nutrition in Sustainable Agriculture

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Abstract: The global demand to enhance agricultural productivity from a steadily decreasing and degrading land resource base is a major strain on agro ecosystems. Current strategy to maintain agricultural productivity is exclusively through inputs of costly chemical fertilizers. Although the use of chemical fertilizers is credited with nearly fifty percent increment in agricultural production but their adverse effects on soil health and environment necessitate the need to adopt sustainable and environment friendly approaches. In sustainable production systems, replenishment of soil nutrients especially phosphorus is a major challenge as it is mainly fertilizer dependent. Phosphorus fixation and precipitation cause its deficiency for plant development and in turn restricts the crop growth. To circumvent the soil P deficiency, an alternative, inexpensive technology in the form of phosphate solubilising microorganism could be exploited while reducing the dependence on expensive chemical P fertilizers. The phosphate solubilizing microorganisms (PSM) inhabiting diverse ecological niche not only function as P provider to plants through inorganic/organic phosphate solubilisation/mineralization processes but also facilitate plant growth by N_2 fixation, enhance availability of other plant nutrients, synthesize phyto-hormones, suppress plant diseases (bio-control) and reduce the toxicity of ethylene through 1-aminocyclopropane-1carboxylate (ACC) deaminase. The challenge is how to make best use of such biological resources to increase the crop productivity. In this chapter, attention is paid to the effective use of phosphate solubilizers for improving plant growth through decreased application of chemical fertilizers.

Key words: Phosphorus, microbial inoculants, crop productivity, plant growth promoting attributes.

Introduction

The first green revolution resulted in global food security and made some of the developing countries like India self sufficient in food. However, the ever increasing population is again a serious threat to our food security. The situation demands second green revolution in the form of 50 % incremental food production in the next two decades. Indian agriculture largely depends upon the use of chemical fertilizers as they provide the plants with desired nutrients. However, the low fertilizer use efficiency demands the input of increased dose of chemical fertilizers to meet the nutritional needs of plants. The use of chemical fertilizer is reaching their maximum theoretical use beyond which, the soils fail to respond to increase crop productivity. The conventional agricultural practices are unable to sustain agriculture production base. There is need for making the soil-plant system healthier to harvest the desired results of increased productivity. After nitrogen, phosphorus is second major nutrient whose low availability is a major constraint limiting the plant growth globally. Despite being abundant in soil, its chemical fixation in the form of $AlPO_4$ and $FePO_4$ under acidic pH and as calcium phosphate in alkaline soil causes its deficiency. The concentration of most minerals in soil solution is in millimoles but phosphorus is available in micromoles only [39]. The organic P also contributes

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largely in organically amended soils. The major portion of it is in the form of phytate that constitutes about 50-70 % of the total organic P content in soil. The persistent application of chemical fertilizers with the aim to increase crop productivity has deteriorated the soil and environmental health. The microbial communities and diversity is also being affected [31]. The long term effect of fertilizer addition has been reported to cause an initial decrease in basal and substrate induced respiration by streptomycin sulphate (fungal activity) and actidione (bacterial activity) and microbial biomass carbon of Pasteur soils [9]. Similarly application of triple superphosphate (94 kg ha^{-1}) has shown a substantial decrease in microbial respiration [12].

The addition of K2PO4 at 25 Kg ha^{-1} and its interaction with upland grass land species (*Agrostis capillaries, Festuca ovina, Lolium perrene*) showed a shift in bacterial and fungal communities of upland soil. The phosphate application may be the important contributor to structural changes of microbial communities during agricultural management [43]. The impact of high use of fertilizer is also being experienced in the form of yield stagnation. Therefore, there is need to find alternative strategies that can ensure competitive yield and maintain the ecological balance of the soil eco-system. The sustainable agriculture system demands the use of environment friendly practices with reduced input of chemical fertilizers and low or no input of pesticides. Emphasis is on the utilization of unavailable P sources where microbial inoculants can improve the P availability to plants. A rational exploitation of soil microbial activities and input of less expensive sources of plant nutrients may help to overcome the low P availability constraints of soil. The organic fertilizers based on plant growth promoting rhizo-bacteria can act as natural stimulator of plant growth and development. Living cell formulations in the form of microbial inoculants or bio-fertilizers improve the soil and plant health by increasing the availability and uptake of nutrients for the plants. They can fulfil the society demands of green technologies in production.

The *in vitro* studies have shown the potential of bacteria, fungi and actinomycetes in liberating the soluble phosphate ions from insoluble inorganic phosphate compounds [19,32]. The fungi have been reported to be more successful due to their competitive saprophytic ability and performance under adverse environmental conditions of low moisture and high temperature and high chemical/ metal concentrations.

Phosphate solubilising microorganisms

Many soil microorganisms have the potential to transform insoluble forms of phosphorus to an accessible soluble form contributing to plant nutrition as plant growth promoting microorganisms. These microorganisms can solubilise and mineralize P from inorganic and organic pools of total soil P and may be used as inoculants to increase P availability to plants [42]. Phosphate solubilising microorganisms are ubiquitous and their population density varies with the type of soil, its nutritional status and environmental factors. A majority of the phosphate solubilising microorganisms have been reported to be present in rhizosphere of leguminous and non leguminous crops, soils of rock phosphate deposit area, glands of *Cassia occidentalis*, in phyllosphere of crop plants, in black and lateritic soils as well as in forest seed beds. The presence of root exudates in the rhizosphere not only helps in fungal colonization but also harbours majority of phosphate solubilising microorganisms in their vicinity due to the availability of carbohydrates, amino acids, organic acids and growth promoting substances. The most efficient phosphate solubilising bacteria belong to genus *Pseudomonas* and *Bacillus*, though species of genus *Burkholderia*, *Escherichia, Micrococcus, Arthrobacter, Methylobacterium, Acremonium* and *Sarcina* have also been reported. The common *Bacillus* species include *B. subtilis, B. circulans, B. brevis, B. megaterium, B. polymyxa, B. mesentricus, B. mycoides and B. pulvifaciens*. The *Pseudomonas sp.* include *P. straita, P. aeruginosa, P. putida, P. vermiculosum and P. fluorescence* [6,18,38,45,61].

The actinobacteria of genera *Kitasatospora* isolated from maize rhizosphere has been reported as new potential phosphate solubilizer [38]. The P solubilising activity of bacteria may be reduced on repeated sub culturing but this may not be the case with fungi that exhibit better P solublizing ability compared to bacteria in solid as well as liquid media [19]. In liquid culture the fungal hyphae have been found to be attached to mineral particles as seen under electron microscope [11]. Moreover, fungi can travel long distance more easily than bacteria and are more important for phosphate solubilisation. The most efficient phosphate solubilising fungi belong to genus *Aspergillus* and *Penicillium,* though sp. of *Cladosporium, Fusarium, Cylindrosporium, Sclerotium, Paecilomyces, Curvularia* and *Mycelia* have also been cited as active phosphate solubilizers [17,30,38,51,59]. Phenotypic mutants of *Aspergillus tubingenesis* obtained by UV irradiation showed its highest ability to solubilise both tricalcium and rock phosphate and mineralize phytate [55]. The plant growth promoting and biocontrol fungus *Trichoderma harzianum* has also been reported as potential solubilizer of rock phosphate [1]. The P solubilization and antifungal activity of *Rhizocotania solani* and *Fusarium oxysporium* suggests their beneficial prospects for improving P nutrition, disease control and crop productivity [52].

The nematofungus *Arthrobotrys oligospora* was efficient in solublizing kodjari rock phosphate (Barkina Faso KRP), Togolese rock phosphate (Senegal TRP) and tilemsirock rock phosphate (Mali TIRP). The fungus was found competent to dissolve additional P from rock phosphate in vivo [14]. The stress tolerant phosphate solubilising microorganisms have been found in non rhizosphre soil, rhizosphere and rhizoplane soils of *Prosopsis juliflora* showing their potential application as microbial inoculants for stressed environment [28].

Inoculation Technologies for phosphate solubilising microorganisms

Microbial inoculants or biofertilizers are the live formulations of the beneficial microorganisms that on introduction to soil, directly or indirectly improve the availability of that nutrient in soil and its uptake by plant. To allow a wider use of bio-fertilizers, the bulk production of pure inocula with high infectivity potential is the major issue. The selection of microbial strain, selection of desired carrier, mixing of inocula with selected carrier and development of microphos inoculants, maintaining its quality standards and design of correct delivery methods are some of stages that need due attention.

Formulations in natural carriers

The microbial strains with high potential to solubilise insoluble P under *in vitro* conditions are used for mass production and their ultimate transfer from lab to land. The cost of production is a major constraint. To ensure the market sustainability, the cost of bio-fertilizer should not exceed that of conventional ones. Therefore, some cost effective carriers such as peat, cow dung cake powder, farm yard manure, lignite, charcoal-soil based carrier have been tested and found useful. However, various amendments to improve their shelf life are continuously being made. Gelatin and soymeal have been found effective in improving the moisture status of charcoal-soil (3:1) carrier thereby improving the shelf life of introduced microorganisms [26]. Spent compost obtained from mushroom cultivation after amendment with charcoal was found to be a suitable carrier for mass multiplication of beneficial microorganism [3]. Adding phosphate solubilising bacteria to vermicompost increased the quantity of available phosphorus in finished product [33,56]. Agro-industrial wastes such as dehydrated powdered bagasse with traces of mahua cake and silk cocoon waste, wheat bran, sugar beet, olive cake, dry olive waste have been explored and found successful in maintaining high population density of phosphate solubilising fungi [17,26,57]. Coal, clay and lapillus, volcanic pumice or diatomite earth available in different regions can also be used as carriers [50]. However, the microbial load in these carriers is relatively lower than in organic carriers. A mixture of organic and inorganic carrier material was found successful in increasing the activity of *Burlholderia* sp. [2]. The survival of inoculated microorganism in these carriers may be affected during transportation, storage and handling, hence needed to improve their stability and shelf life.

Polymer based carriers

Organic polymers (polysaccharides) are the compounds that in presence of ions form cross links that create a complex structure. The polymers immobilize the microorganisms in the matrix and release them slowly via degradation process [36]. The polymer based formulations have certain advantages as providing protection against environmental stress and maintaining a longer shelf life of inoculated strain even at room temperature. However, the storage at low temperature further prolongs the shelf life of encapsulated microorganisms [7].

The most commonly used polymer for cell encapsulation includes alginate. It is a polymer of D-mannuronic acid and L-glucuronic acid. *Macrocystis pyrifera* and *Sargassum sinicola* are the two algae that are known to produce alginate [62]. When alginate solution is dropped in to the cation solution beads of calcium alginate are formed with diameter ranging from 2-3 mm. The micro beads with size of 50-200 µm have been reported to entrap microbial population up to 10^8-10^9 CFU g^{-1} [8]. Though, alginate beads can maintain shelf life of immobilized cells for several months but improving the viability of living cells by supplementing inoculums with skim milk has also been studied [24]. The chitin filled beads maintained the better porous structure of the beads compared to starch filled beads [54]. Moreover, the bacterial efficacy was also more in soil-plant system. Calcium alginate was found superior to charcoal soil based carrier for maintaining the higher population of *Pseudomonas striata* and *Bacillus polymyxa* [58]. However, incorporation of charcoal-soil adversely affected the initial loading of these organisms in alginate gel. Alginate alone maintained higher population of these organisms at a higher storage temperature of 40°C. Encapsulation of bacteria in alginate, standard starch and modified starch mixture improved the physical characteristics of beads as well as the cost of production was lowered [25].

Biofilms as a carrier

Biofilms of beneficial microbial flora can be formed by growing them on inert support as charcoal, resin, concrete, clay brick and sand particles. Biofilms grow around the particles and size of the biofilm particles gradually grows to several mm. Such biofilms are called granular biofilms [41]. Biofilms are formed more readily in presence of phosphorus that increases the adhesion ability of the cells. Biofilms developed under *in vitro* cultures containing both fungal and bacterial strains help the microorganisms to survive even under stress conditions.

Silica has been found to be a promising host for encapsulation of beneficial m.o under polymer based carriers. Bacteria can either be trapped into alginate micro beads coated with silica membranes or into macro cavities created inside the silica matrix. Silica improves the mechanical properties of alginate beads, reduces the cell leakage and enhances the cell viability [10].

Nanoformulations

Application of bio-nanotechnologies can also open new avenues for preparing carrier based inoculants [40]. Nanoparticles of particular dimension are developed from organic and inorganic materials. The stability of bio-fertilizers and bio-stimulators in terms of desiccation, heat and UV inactivation may be enhanced through nano formulations. The same procedure can be employed for harvesting microbial cells from fermentation processes and applied to plant system.

Inoculation effect of phosphate solubilising microorganisms on crop productivity

In vitro solubilisation studies of phosphate solubilising microorganisms led the researchers to use them as bio-inoculants to release P from soil reserves and improve its uptake by plants. These microbes perform important functions in agro-ecosystems including their role in plant growth promotion through mineral nutrition. The PSM can be used for all the crops irrespective of them being cereal or leguminous or vegetable crops. These inoculants being eco-friendly provide yield increment with low input cost. A substantial increase in plant growth due to single or dual association of rhizospheric microbial flora has been reported by Zaidi and Khan [63]. The integrated use of rock phosphate and phosphate solubilising cultures are known to add 30-35 Kg P_2O_5 ha^{-1}. The promotion of overall performance of plant grown in different agro-ecosystem in response to PSM inoculation is discussed.

Paddy

PSB inoculation in conjunction with bone meal application to soil not only improved the nitrogen and phosphorus uptake of plants but also the grain yield [48]. On the contrary, culture filtrate of phosphate solubilising bacteria did not exert any significant effect on the germination of paddy seeds but the secondary root development was improved. The use of *Bacillus* sp. as microphos inoculants besides improving the P uptake by paddy plants also improved the biomass production of rice plants [5,13]. Mixed culture of *P. striata* and *A. awamori* with and without addition of chemical fertilizer resulted in an appreciable increase in yield and nutrient uptake by rice [35] compared to single inoculation. Inoculation of *Bacillus polymyxa* exerted positive effect on crop growth of low land paddy whereas, in non- acidic sols, *P. striata* was more superior [23]. Pre plant inoculation of rice seedlings-roots with *A. awamori* resulted in an increase of 0.09-0.22 t ha^{-1} [15]. The agronomic efficiency increased with initial application of rock phosphate.

Wheat

Use of Fosfo 24 and PSB increased the yield of wheat by 7-33 %. The seed inoculation with *B.megaterium* var phosphaticum and *B.circulans* also resulted in improved P uptake by wheat plants [53]. Gaur [22] reported that inoculation with *P. striata and A.awamori* enhanced the wheat yield by 10-20 % when phosphate solubilising bacterial (PSB) cultures were added to soil in conjunction with FYM. Addition of rock phosphate to soil along with PSB inoculants improved P uptake in neutral to alkaline soils [5]. *A.niger, A. fumigatus* and *P. pinophilum* increased the yield components of wheat significantly. *P. pinophilum* improved the wheat grain yield by 28.9 and 32.8 % in presence of rock and superphosphate respectively [60]. A 64 % increase in P uptake by wheat plant has been observed due to incorporation of rock phosphate in conjunction with *P. fluroescence* and *Serratia sp*. [46].

Maize

Seed inoculation of maize with *Pseudomonas* sp. increased the grain yield and P uptake by maize compared with uninoculated control [29]. In vertisol amended with cotton stalk and rock phosphate along with *P. striata and B. polymyxa* inoculation increased the size and weight of ear head, number of spikelets per ear, straw and grain yield as well as nutrient uptake in sorghum crop [27]. Inoculation of *P. oxalicum* CBPS-3F-Tsa, alone or in combination with rock phosphate increased the growth and P uptake in maize plants compared with uninoculaed control [49].

Pearl millet

Seed inoculation of *P. striata* and its application to soil fertilized with low-grade Mussoorie rock phosphate @ 60kg P_2O_5 ha^{-1} under pearl millet (*Pennisetum glaucum*) not only improved the root and shoot biomass (2.01 and 29.12 g pl^{-1}) respectively, but also increased the availability of soil P (43.44 µg g^{-1}), and resulted in higher P uptake by straw and grain. Its application to soil in presence of rock phosphate provided an option to reduce the input of costly inorganic P fertilizer, as the crop yield in the presence of RP_{60}+ *P. striata* was comparable with low dose of super phosphate (SP_{30}) + *P. striata*. The microphos inoculants developed from the bacterial strain with multiple functional attributes may help the marginal farmers replenish the soil P more economically compared with costly inorganic phosphate fertilizer [16].

Soybean

The grain yield of soybean has been improved by 2-4 q ha^{-1} when rock phosphate was used along with *P. striata* whereas increase was hardly 1 q ha^{-1} with 80 Kg P_2O_5 ha^{-1} as super phosphate [21]. Phosphate solublilizing *B. firmus* capable of producing indole acetic acid was found to improve the available P in soil and vegetative growth of soybean but did not improve the grain yield of soybean [4]. Soil tagging with soybean as test crop revealed that application of untreated rock phosphate is of little significance from dry matter yield and P uptake parameters point of view. Use of rock phosphate-pyrite mixture, microbial inoculants and rock phosphate slurry mixture enhanced the percent P utilization and dry matter yield significantly over rock phosphate alone [47]. *P.vermiculosum* and rock phosphate resulted in highest available P of soil. The grain yield was also more with this culture compared to *P. striata* or *A. awamori* and rock phosphate. The positive effect of *P. striata* in improving the yield and nutrient uptake of soybean was also recorded in presence of fly ash [20]. However, the uptake of micro-nutrients did not improve. Application of fly ash at 40 t ha^{-1} + *P. striata* resulted in the statistically significant increase in P uptake.

Chickpea

The inoculation of chickpea seeds with *P. striata, B. polymyxa* and *A. awamori* increased the grain yield of crop by 5.4, 7.4 and 7.0 q ha^{-1} respectively over control. However, the increase in yield due to application of rock phosphate without PSM was only 1.9 q ha^{-1} [22]. The seed inoculation with thermotolerant phosphate solubilizng microorganisms viz. *B. circulans, B. subtilis* and *A. niger* improved nodulation, available P_2O_5 content of soil, root and shoot biomass, straw and grain yield and uptake of N and P by mung bean. Rock phosphate at 40 kg P_2O_5 ha^{-1} coupled with PSB gave results comparable to SP_{20} + PSB [19]. Growth and yield parameters of chick pea improved due to inoculation of Trichoderma spp [44]. Mittal *et al* [37] observed the effect of two strains of *A. awamori* and four strains of *P. citrinum* on growth and seed production of chickpea. Maximum inoculation effect in the form of 7-12 % increase in shoot height, three fold increases in seed number and two fold increases in seed weight were recorded with *A. awamori*. All the cultures produced plant growth promoting hormones.

Vegetable crops

The potato yield in sandy loam alluvial soil showed an increase of 60, 52 and 16 % when tubers were treated with *P. striata, B. polymyxa* and *A. awamori* respectively**.** The yield so obtained was statistically comparable to 70 % in tuber yield with superphosphate SP_{80} as P_2O_5. However, in hilly soils, *P. striata* inoculation resulted in an increase of 23 q ha^{-1} [34]. The combined inoculation of *P. striata* and *B. polymyxa* increased the tuber yield by 35.2 % whereas single inoculation enhanced the yield by 30.8 and 23 % respectively.

Conclusions

The utilization of natural resources in the form of phosphate solubilising microorganisms has a huge potential for improving the P nutrient availability, plant productivity and protection of agro ecosystem from the excessive use of chemical phosphate fertilizer. However, selection of the most efficient inoculants strain and development of formulation requires extensive research efforts for their application and success under field condition to benefit the marginal farmers.

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