Microbial mobilization of soil phosphorus and sustainable P management in agricultural soils

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Phosphorus plays a vital role in maintaining soil fertility and securing global food supply by being crucial for plant, human and animal life. Globally phosphorus is mined from geological sediments and most of the mined P is added to agricultural soils to meet the critical need of crop plants for agronomic productivity. However, recovery of P by plants is abysmally low and major amount of added P is fixed in the soil creating a need for addition of P fertilizer. Microorganisms play a fundamental role in mobilizing inorganic and organic P in the soil and the rhizosphere. Wide variety of bacteria, fungi and endophytes solubilizes insoluble P through the production of organic acids, a feature which is genetically controlled and can be suitably manipulated to produce efficient transgenic strains. Plant inoculations with phosphate solubilizing microorganisms (PSMs) during field studies, however, had inconsistent effect on plant growth and crop yields due to variations in soil, crop and environmental factors affecting the survival and colonization of the rhizosphere. Increasing availability of soil P through microbial inoculation will necessitate identification of the most appropriate strains, preparation of effective formulations, and introduction of efficient agronomic managements to ensure delivery and survival of inoculants and associated improvement of P efficiency.

Keywords: Direct oxidation pathway, genomics of MPS, microbial phosphate solubilization, sustainable P management, transgenic P-solubilizers.

PHOSPHORUS, the macronutrient next only to nitrogen in its importance to life forms, plays a stellar role in the transfer of energy, cellular metabolism including nutrient uptake, and preservation of genetic information. In fact, no life process can function without this element. Phosphorus which is commonly available in the environment in the fully oxidized state as phosphate consistently forms insoluble chemical complexes with calcium, iron and aluminium making it unavailable for uptake by plants. Phosphorus availability in several soils is ~1 μmol l^{-1}, but for optimum productivity, P-requirement for plants is ~30 μmol l^{-1}. It is now well-established that P-availability in soils is a major factor restraining plant productivity[1]. Phosphorus limitation in terms of incidence and relative enormity affecting primary productivity are approximately equal in both terrestrial and freshwater ecosystems. In terrestrial systems, P limitation develops because a large amount of the soil P is mostly unavailable to growing plants. Replenishing soil P to enhance and maintain productivity has long been recognized by agronomists in P-deficient agricultural soils. Application of phosphate fertilizers, therefore, has been considered vital for achieving economic yield in many agro-ecosystems. As a result, during the last century, P from geological reserves has been mobilized to a great extent for fertilizer production. Indeed, it is assessed that anthropogenic activities have amplified global P cycling by ~400% relative to pre-industrial times, several folds higher than carbon (~13%) or nitrogen (~100%)[2].

P availability and dynamics in soils and rhizosphere

Globally, phosphorus needs are currently met from geological sedimentary rock formations available in select areas of the world. Rock phosphate mining in 2011 amounted to 191 metric tonnes (mt)[3], corresponding to 25 Tg P $year^{-1}$. Most of the P mined (18 Tg P $year^{-1}$) is added to agricultural soils as P-fertilizer[4] and a small quantity used in the detergent and food industry. Overall, 82% of P is required for fertilizers, 7% as a nutrient in feedstock and 11% for pharmaceuticals and industry. Estimates vary as to the total P reserves available globally[3,5,6]. The future demand of P is anticipated to increase at 2.3% p.a. largely due to increasing food demand in the developing world, a shift towards meat-based diet (which consumes more P than vegetarian diet) and increasing biofuel production. Concerns of future demand–supply gaps for P emerged on its availability, with the assumption that the available global reserves could be depleted

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in 50–100 years hence and the global peak in P production is predicted around 2030 (refs 3, 7). However, revised analyses increased the estimated global P reserves from 15 Gt in 2008 to 71 Gt in 2011 (refs 6, 8) which is expected to last 300 years. The main issues of concern are the likelihood of quality deterioration of rock-phosphates and increase in the cost of P extraction over a period of time, apart from geopolitical factors and the potential for monopoly pricing.

Phosphorus exists in soils in both organic and inorganic forms. Phosphorus fertilizers are the main input of inorganic P in agricultural soils and approximately 70% to 80% of P in cultivated soils is inorganic[9]. Phosphorus in fertilizers is converted to water-soluble Pi as orthophosphate ions $H_2PO_4^-$ and HPO_4^{2-} within hours after application to soil[10]. As the fertilizer reaches the soil, available soil moisture begins to dissolve the fertilizer particles increasing concentration of Pi in solution and diffuses at short distance from the fertilizer particles[11]. Inorganic phosphorus is negatively charged and readily reacts with iron (Fe^{3+}), aluminium (Al^{3+}), and calcium (Ca^{2+}) ions to form relatively insoluble complex becoming unavailable for crop uptake and is considered fixed. The solubility of various inorganic phosphorus compounds is influenced by the soil pH with soil phosphorus being most available for plant use at pH values of 6 to 7. When pH values exceed 7.3, phosphorus is made unavailable by fixation as calcium phosphates while at pH less than 6, phosphorus is fixed up in aluminium phosphates and at pH below 5, phosphorus is fixed as iron phosphates.

A second major part of soil phosphorus is present in the organic form, largely as inositol phosphate (soil phytate), which accounts for ~50% of the total organic P. Organic-P forms are mostly found in humus and other soil organic components. Phosphorus present in soil organic fractions is released by mineralization engaging soil microorganisms and is highly influenced by soil moisture and temperature. Besides, large quantities of xenobiotic phosphonates are also released into the environment. Despite being rich in phosphorus, the concentration of soluble P (i.e. bio-available P) is usually very low in soils – 0.05% of the total P content of which only 0.1% is plant available.

Phosphorus being lowly soluble and mobile in soil, it can be rapidly consumed in the rhizosphere by plant uptake, effecting a P concentration gradient in a radial direction away from the root surface. Although the total P content of the soil usually exceeds the plant requirements, availability of P to plants could be restricted by low mobility of soil P. Accordingly to meet the plant demand, soluble P in the rhizosphere soil solution should be replaced several times a day by transfer from bulk soil to the rhizosphere[12]. Phosphorus dynamics in the rhizosphere is mainly influenced by root growth and activity, and also highly affected by physicochemical properties of the soil[13]. Biochemical processes operating in the rhizosphere not only determine mobilization and acquisition of soil nutrients as well as microbial dynamics, but also oversee nutrient-use efficiency of crops, and thus greatly influence crop productivity[12–15].

Microorganisms affecting P release in soils

Using microbes to improve mobilization of lowly available forms of soil P is not so novel a concept[16]. Number of soil microbes have been identified to solubilize insoluble P-complexes into solution making it possible for its uptake by plants[17]. Several species of fungi and bacteria, commonly known as phosphate-solubilizing microorganisms (PSMs) help the plants in mobilizing insoluble forms of phosphate. PSMs improve the solubilization of phosphates fixed in soil resulting in their uptake by plants and higher crop yields, and are used as biofertilizers. Significant increase in grain yield was reported for rice, chickpea, lentil, soybean and cowpea when *Pseudomonas striata*, *Aspergillus awamori* and *Bacillus polymyxa* were used either singly or in combination[18]. Several bacteria, fungi including mycorrhizal fungi and actinomycetes are highly capable of converting insoluble phosphate into soluble inorganic phosphate ion (Table 1).

Microbial strategy for release of unavailable forms of P

Phosphate-solubilizing bacteria employ different strategies to convert unavailable forms of phosphate into available forms. In most bacteria, production of organic acids is shown to be related to the dissolution of mineral

Table 1. P-solubilizing microorganisms

Bacteria	*Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, *Enterobacter* sp.[66] *Beggiatoa*, *Thiomargarita*[67] *Leifsonia xyli* FeGl 02, *Burkholderia cenocepacia* FeSu 01, *Burkholderia caribensis* FeGl 03, *Burkholderia ferrariae* FeGl 01. sp.[68].
Actinobacteria	*Actinobispora yunnanensis*, *Actinomodura citrea*, *Microtetrospora astidiosa*, *Micromonospora echinospora*, *Sacchromonospora viridis*, *Saccharopolyspora hirsute*, *Streptomyces albus*, *Streptoverticillium album*, *Streptomyces cyaneus*, *Thermonospora mesophila*[69].
Fungi	Belonging to genera *Aspergillus* (*A. awamori*) and *Penicillium* (*P. bilaii*).
Mycorrhiza	Belonging to genera *Glomus*, *Funneliformis*, *Rhizophagus*, *Sclerocystis*, *Claroideoglomus*, *Gigaspora*, *Scutellospora*, *Racocetra*, *Acaulospora*, *Entrophospora*, *Pacispora*, *Diversispora*, *Otospora*, *Paraglomus*, *Geosiphon*, *Ambispora*, *Archaeospora* sp.
Endophytes	Bacteria: *Achromobacter*, *Acinetobacter*, *Enterobacter cloacae*, *Pantoea agglomerans*, *Pseudomonas* sp.[70]. Fungi: *Piriformospora indica*[71], dark septate endophytes belonging to Ascomycota[72].

phosphates. Goldstein[19] proposed direct oxidation of glucose to gluconic acid (GA) as the foremost mechanism for mineral phosphate solubilization (MPS) in gram-negative bacteria. Organic acids released by the microorganisms act as good chelators of divalent cations of Ca^{2+} coupled with the release of phosphates from insoluble complexes[18]. Organic acids may also form soluble complexes with metal ions co-complexed with insoluble P, thereby releasing the P moiety. Many of the PSMs cause a reduction in the pH of the medium either by H^+ extrusion or by secretion of various organic acids[18–20]. Proton transport from the cytoplasm to the outer surfaces of the microbes may take place in exchange for a cation (especially ammonium) or with the help of ATPase (ABC transporter) located in the cell membrane and uses the energy from ATP hydrolysis[18].

Biochemical mechanism of P release

The 2-keto gluconic acid produced from direct oxidation of glucose by MPS bacteria play an important role in weathering and solubilization of phosphates in soil. Highly efficient solubilization of rock phosphate by *Erwinia herbicola* and *Pseudomonas cepacia* is the result of gluconic ($pK_a \sim 3.4$) and 2-keto gluconic acids ($pK_a \sim 2.4$) formed by direct oxidation of glucose[18]. Some bacteria undertake the direct oxidation pathway to such elevated levels that externally added glucose is quantitatively converted to gluconic acid at concentration of 1 mol l^{-1} or higher. Gram-negative bacteria are more efficient at dissolving mineral phosphates when compared to gram-positive bacteria because of the release of several organic acids into the extracellular medium[18]. Thermotolerant acetic acid producing *Acetobacter* and *Gluconobacter* also have the direct oxidation pathway with thermotolerant glucose dehydrogenase (GDH) and solubilize mineral phosphate.

Apart from gluconic acid, several other organic acids such as acetic, lactic, malic, succinic, tartaric, oxalic and citric acids are also produced (Table 2). Weak organic acids, viz. malate, acetate and succinate are present in the rhizosphere as fermentation products of rhizobacteria. *Pseudomonas* sp. is known to preferentially utilize these weak organic acids over glucose, sucrose and fructose[21]. Similar catabolite repression of glucose metabolism is found in root nodule bacteria[22]. Many fluorescent pseudomonads are also known to solubilize mineral phosphates by secretion of gluconic acid[23]. Presence of malate and succinate has been shown to repress MPS phenotype in fluorescent pseudomonads[24]. Similarly, MPS phenotype mediated by oxalic acid in *Klebsiella pneumonia* is repressed by the presence of succinate[25]. However, the MPS phenotype to be very effective under field conditions would require higher amounts of stronger acids.

The enzyme glucose dehydrogenase (GDH), the key enzyme in the conversion of glucose to gluconic acid, is a quinoprotein that uses the redox cofactor 2,7,9-tricarboxyl-1 H-pyrrolo [2,3-f] quinine-4.5-dione (PQQ)[26]. GDH requires PQQ and has binding sites for Mg^{2+} (*in vitro*), Ca^{2+} (*in vivo*), ubiquinone and the substrate glucose. Two types of GDH have been identified, GDH A and GDH B, based on their localization within the cell. GDH B is soluble (s-GDH) and is reported only from *Acinetobacter calcoaceticus* while GDH A is more widespread and is a membrane-bound enzyme (m-GDH)[18]. PQQ-dependent GDH is present in several bacterial species. While *P. aeruginosa* produces the cofactor PQQ, others such as *E. coli* are unable to produce PQQ and require external supply of PQQ for GDH activity. Location of the GDH apoenzyme on the periplasmic space facilitates binding of PQQ to form the holoenzyme[18]. A soluble NADP-dependent GDH was identified from *Gluconobacter oxydans* along with PQQ-dependent GDH[27].

GDH characterized from various bacteria are about 88 kDa monomeric proteins having primary structure similar to each other, differing marginally in some of the properties such as substrate specificity[28]. The enzyme has an N-terminal hydrophobic domain (residues 1–150) consisting of five transmembrane segments ensuring a strong fastening of the protein to the membrane, and a large conserved PQQ-binding C-terminal domain with the catalytic activity. The ubiquinone-binding site and also a membrane-binding site were demonstrated to be located in the large C-terminal domain. The N-terminal domain interacts with C-terminal domain via domain–domain interaction, stabilizes the GDH and also a potential signal sequence for the C-terminal domain[18,28].

Genomics and proteomics of P solubilizing enzyme

The MPS characteristic is induced or repressed by the levels of inorganic phosphate available in the environment. A cosmid library of *E. herbicola* in *E. coli* screened for MPS phenotype resulted in the isolation of a recombinant clone that exhibited either induction or repression in the presence or absence of soluble or insoluble form of phosphate levels. It suggests that these genes play a pivotal role in bacterial phosphate starvation metabolism and are overseen by catabolic repression-like behaviour[18,29]. Gene responsible for MPS ability in *E. herbicola* showed similarity to gene III of *pqq* gene cluster from *A. calcoaceticus*, and to *pqqE* of *K. pneumonia* and it enabled *E. coli* HB101 to solubilize hydroxyapatite[29,30]. Although *E. coli* does not possess cofactor *pqq* synthesis genes, some *E. coli* strains indicated possession of cryptic *pqq* biosynthesis genes based on the observation that single open reading frame (ORF) of *E. herbicola* PQQ synthase gene could complement the cofactor requirement and demonstrate the MPS phenotype. Similarly, 7.0 kb genomic DNA fragment of *Rahnella aquatilis* conferring hydroxyapatite solubilization in *E. coli* showed two complete ORFs and one partial ORF showing similarity to *pqqE* of

Table 2. Individual or *pqq* gene clusters from P-solubilizing bacteria cloned and expressed in *E. coli*

Gene/function	Source	Host	Mineral P solubilized	Organic acid	Reference
PQQ biosynthesis	*Serratia marcescens*	*E. coli*	TCP	GA	Krishnaraj and Goldstein[33]
pqqE	*Erwinia herbicola*	*Azospirillum* sp.	TCP	GA?	Vikram *et al.*[73]
pqqED genes	*Rahnella aquatilis*	*E. coli*	HAP	GA	Kim *et al.*[31]
Unknown	*Enterobacter agglomerans*	*E. coli*		GA?	Kim *et al.*[34]
pqqABCDEF genes	*Enterobacter intermedium*	*E. coli* DH5α	HAP	GA	Kim *et al.*[74]
Ppts-gcd, *P gnlA-gcd*	*E. coli*	*Azotobacter vinelandii*	TCP	GA	Sashidhar and Podile[18]
gabY Putative PQQ transporter	*Pseudomonas cepacia*	*E. coli* HB101		GA	Babu-Khan *et al.*[32]
Unknown	*Erwinia herbicola*	*E. coli* HB101	TCP	GA	Goldstein and Liu[19]
gltA/citrate synthase	*E. coli* K12	*Pseudomonas fluorescens* ATCC 13525	DCP	Citric acid	Buch *et al.*[72]
Unknown	*Synechocystis* PCC 6803	*E. coli* DH5α	RP	Unknown	Gyaneshwar *et al.*[39]
gad/gluconate dehydrogenase	*P. putida* KT2440	*E. asburiae* PSI3	RP	GA and 2KG	Kumar *et al.*[35]

E. herbicola, *K. pneumoniae*, *A. calcoaceticus* and *pqqC* of *K. pneumoniae* respectively[31].

Many individual or *pqq* gene clusters from P solubilizing bacteria were cloned and expressed in *E. coli* which enabled them to produce GA leading to MPS phenotype (Table 2). Expression of *gabY* (396 bp) gene in *E. coli* JM109 induced MPS ability and GA production. *gabY* gene sequence was similar to membrane-bound histidine permease component which may be a PQQ transporter[32]. *Serratia marcenses* genomic DNA fragment induces GA production in *E. coli* but it does not show any homology with either *gdh* or *pqq* genes[33]. The DNA fragment did not confer GA secretion in either *E. coil gdh* mutant or in other *pqq* producing strain. Thus, it was postulated that the gene product could be an inducer of GA production. Similarly, other reports showed genes that are not directly involved in *gdh* or *pqq* biosynthesis could induce MPS ability. Genomic DNA fragment of *Enterobacter agglomerans* showed MPS ability in *E. coli* JM109 without any significant change in pH[34]. MPS genes from *R. aquatilis* showed higher GA production and hydroxyapatite dissolution in *E. coli* compared to native strain[31]. Overexpression of *P. putida* KT 2440 gluconate degydrogenase (*gad*) operon in *E. asburiae* PSI3 improved MPS phenotype by secretion of 2-ketogluconic acid along with GA[35].

Glucose dehydrogenase (ES chimera) encoding the N-terminal transmembrane spanning PQQGDH region of *E. coli* and the C-terminal periplasmic domain from *S. marcescen*s GPS5 was constructed and its expression was studied[36]. Four different mutants (E742K, Y771M, H775A and EYH/KMA) of this chimeric GDH exhibited alteration in the substrate affinity, EDTA and temperature tolerance. Similarly, chimeric GDHs to improve EDTA tolerance, thermal stability, substrate specificity and stability of cofactor PQQGDH from *E. coli* and *A. calcoaceticus* have also been extensively studied. A chimeric PQQGDH with 97% of N-terminal region of *E. coli* PQQGDH and 3% of *A. calcoaceticus* PQQGDH was constructed for increased thermal stability[37]. Other multichimeric constructs with varying N-terminal and C-terminal regions of *E. coli* and *A. calcoaceticus* identified the regions responsible for EDTA tolerance to be located between 45% and 56% of the distance from the N-terminal region of *A. calcoaceticus* PQQGDH, corresponding to about 90 amino acid residues[18]. Based on the sequence homology of the C-terminal catalytic domain (151–796 amino acid residues), the 3D structure of *E. coli* GDH has been constructed[38]. When validated using the Ramachandran plot, geometrical parameters of the model revealed 95.8% of residues in the allowed regions and 2.2% of the residues in disallowed regions. From the model 5, different amino acids have been identified that are specifically involved in maintaining the right configuration of PQQ along with a Ca^{2+} ion in the active site. Two amino acids, Asp-204 and Gly-776 have been found to be highly conserved on the surface of the protein that might be involved in ubiquinone binding or transfer of electrons to the ubiquinone[38].

Factors influencing the efficacy of P release by microorganisms in soils

Field studies of plant inoculations with PSMs had inconsistent effect on plant growth and crop yields[39,40]. This has been attributed to variations in soil, crop and environmental factors influencing the survival and colonization of the rhizosphere. Generally, microbial population size decreases rapidly after inoculation in soil[41,42]. Survival depends upon the abiotic, biotic factors, soil composition[43], temperature, moisture, carbon status[40] and presence of recombinant plasmid[42]. Biotic factors also play an important role in the survival of the inoculated strains as decline was observed in non-sterile soil which is minimal in sterile soils[44]. Effectiveness of introduced microorganism in the field condition requires maintenance of minimal number in soils. Increased population of inoculated microbes was observed in sterile soil[45]. In case of *Rhizobia*, 300 cells per seed are sufficient for optimal nodulation[46].

Root colonization ability. Root colonization is a major factor influencing the success of inoculants. Majority of the microbial population found in the soil are associated

with the plant roots where their population can reach up to 10^9 to 10^{12} per gram of soil[47], leading to biomass equivalent to 500 kg ha^{-1} (ref. 48). Abundance of microbes in the rhizosphere is due to secretion of high amount of root exudates[49]. Root-associated bacterial diversity and their growth and activity vary in response to the biotic and abiotic environment of the rhizosphere of the particular host plant[50]. Rhizobacteria interaction with plant root is mediated by secreted compounds (signals and nutrients) that vary in quantitative abundance and qualitative diversity depending upon the characteristics of the particular host[51,52].

Soil properties. Soil properties vary in term of texture, particle and pore size of soil. Pore size distribution determines the efficacy of the microorganism and different behavioural pattern in bacteria when released in different texture soil can be correlated by protective pore spaces present in soil[42]. A three-year study in loamy sand and silt loam soils showed that survival of inoculated *P. fluorescens* was better in finer-texture soil, i.e. silt loam than in the sandy soil[53].

Abiotic stresses. PSMs need to survive a variety of abiotic stresses under field conditions which depend on the agro-climatic conditions along with seasonal changes. Many PSMs have been isolated which could retain their ability under different stress conditions[54]. Efficiency of PSMs differs significantly with high or low temperatures. PSMs in tropical countries need to tolerate 35–45°C temperatures, while temperate regions require cold tolerance. Many *Bacillus*, *Streptomyces* and *Aspergillus* strains showed very good P solubilization ability at 50°C, which could facilitate composting[55]. *Acinetobacter* CR 1.8 could grow up to 25% NaCl, between 25°C and 55°C and at pH 5–9, but maximum solubilization of tricalcium phosphate and aluminium phosphate was obtained at neutral pH, and 37°C (ref. 56). In contrast, many PSMs are known to solubilize P at low temperature. A mutant of *Pseudomonas fluorescens* could solubilize mineral phosphates to similar extent at both 10°C and 25°C (ref. 57). *Pseudomonas corrugate* mutants were isolated with P solubilization ability at 4°C as well as at 25°C (ref. 58). Bacteria isolated from cold conditions of the Himalaya demonstrated good P solubilizing ability at low temperature[23]. Halophilic P-solubilizing *Kushneria* sp. YCWA18 isolated from Daqiao Saltern on the coast of Yellow Sea of China, could grow rapidly at 28°C and the concentration of NaCl was 6% (w/v)[59]. Thus, many PSMs can tolerate many stress conditions but very few can retain the phenotype under stress conditions.

Substrate availability. Substrate availability often limits the performance of the inoculants. Amount of carbon used in the laboratory studies (several mg per gram soil) are very high compared to carbon present in the soil and rhizosphere[60]. Plant roots secrete complex mixture of organic compounds, viz. organic acid, amino acid and sugars. Sugars secreted are glucose, fructose, maltose, ribose, sucrose, arabinose, mannose, galactose and glucuronic acid[51,52]. Organic acids secreted in rhizosphere are malate and citrate, releasing P from soils. Among amino acids, histidine, proline, valine, alanine and glycine are present[61].

Field studies on commercial exploitation of microbes for P release in soils

Inoculation of PSMs has revealed that they not only improve the availability of soluble P to plants but also facilitate the biomass and yield of different agronomically important crops by diverse mechanisms. Most of the reports on PSMs have shown that they possesses multiple plant growth promoting traits such as production of indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and siderophores in addition to gluconic acid production. Application of PSMs can increase plant growth, grain yield and also protein content[42]. Arbuscular mycorrhiza and PSB isolated from composts and macrofauna showed plant growth promotion and enhanced mycorrhizal colonization[43]. Field studies of plant inoculations with PSMs, however, had inconsistent effect on plant growth and crop yields[39,40]. This has been attributed to variations in soil, crop and environmental factors influencing the survival and colonization of the rhizosphere. Generally, microbial population size decreases rapidly after inoculation in soil[41]. Performance of PSMs on different crop growth and yield data is given in Table 3.

Sustainable P management in soils with apparent P deficiency

Availability of accumulated soil P to plants is largely influenced by the activity of soil microorganisms through their ability to solubilize and mineralize inorganic and organic soil P fractions. Microbially mediated transformation of major soil P fractions is very important to the soil P cycle. The soil microbial biomass contains a substantial pool of immobilized P that is potentially available for plant nutrition. Strategies for utilizing soil microorganisms to improve phosphorus availability include the introduction of mycorrhizal fungi, inoculation of soil-grown plants with P-solubilizing bacteria or fungi, and inoculation with P-mineralizing microorganisms. Role of mycorrhizal fungi in sustainable P management is discussed in detail by Bagyaraj *et al.* (p. 1288) in this issue.

Bio-inoculants have often been projected as vital constituents of integrated nutrient management approaches with specific interest in their potential of solubilizing sparingly available P in order to increase its availability for the crops[18,62]. Research on bio-inoculants has focussed

Table 3. Effect of phosphate solubilizing microorganisms on crop growth and yield

Name of crop	PSM	Crop growth parameter	Increase in yield (%)	Nutrient uptake	Reference
Ground nut	*Aspergillus niger*, *Penicillium notatum*	Dry weight of plant	105	P, N	42
Ground nut	*Aspergillus niger*, *Penicillium notatum*	Protein content	57.5	P, N	42
Ground nut	*Aspergillus niger*, *Penicillium notatum*	Oil content	29.5	P, N	42
Maize	*Penicillium bilaii and Penicillium* spp	Maize yield	20–23	P	44
Wheat	*Aspergillus awamori*	Grain yield	57.25	P, N	45
Tea	*Potassium solubilizing bacteria*	Total yield	75	P, K	46
Maize	*Pseudomonas* sp. *CDB 35*	Grain yield	85	P	47
Sugar cane	*Bacillus megatherium*	Cane yield	12.6	P	48
Kalmegh (*Andrographis paniculata*)	*Trichoderma harzianum* + AM (*Glomus mosseae*)	Overall plant growth	49.8	P	49
Wheat	AM fungi	Shoot dry matter yield	52%	P	50
Wheat	AM fungi	Seed grain spike number	19%	P	50
Wheat	AM fungi	Grain yield	26	P	50
Rice	*Bacillus coagulans*	Grain yield	7593.7 kg /h	P	51
Wheat	*Pseudomonas* and *Bacillus*	Grain yield	2135 kg/h	P	52
Soybean	*Bradyrhizobium japonicum* and PSB	Grain yield	33	P, N	53
Rice	PSB	Grain yield	1–11	P	54
Wheat and bean	*Penicillium bilaii* with VAM fungi	Grain yield	18	P	55
Sun flower	*Bacillus M-13*	Seed yield	15	P	56
Sun flower	*Bacillus M-13*	Oil yield	24.7	P	56
Sugar beet and barley	*Bacillus* sp.	Yield	20.7–25.9	P	57
Chickpea	*Ps. jessenii* and *Mesorhizobium ciceri*	Seed yield	52	P	58
Canola	PSB and *Thiobacillus* sp.	Total yield	60	P	59
Canola	PSB and *Thiobacillus* sp.	Oil yield	39	P	59

largely on introducing free-living microorganisms: (i) Form non-specific beneficial associations with a wide variety of plant hosts. (ii) Can be produced in bulk. Have potential to dwell in the rhizosphere. (iii) Bacteria (mostly *Bacillus* and *Pseudomonas*) and fungi (*Penicillium* sp. and *Aspergillus* sp.) have been identified based on their ability to solubilize orthophosphate from inorganic and organic substrates under laboratory conditions by releasing organic anions, protons, phosphatases and cation-chelating compounds[62].

Opportunities exist to develop novel 'multifunctional' microbial strains as bio-inoculants, such as P solubilizing and N-fixing strains of *Mesorhizobium mediterraneum*[63] and disease biocontrol strains of *Trichoderma harzianum* with capacity to solubilise P[64]. Options for developing transgenic mineral phosphate solubilizing bacteria with high potential of P solubilization is another putative approach[38]. Similarly, increased plant nutrition and growth may be accomplished by using consortia of plant growth promoting rhizobacteria (PGPR)[12,14]. Although, positive responses to such non-symbiotic microbes are often noticed in controlled laboratory and greenhouse experiments, they are mostly non-consistent under field conditions. Analyses of results from 26 field sites in Canada between 1989 and 1995 indicated that inoculation with *P. bilaii* increased in P uptake and yield of spring wheat in only 5 of 47 trials, despite 33 of the trials showing responses to P fertilizer[65]. Although in recent times, formulation and application technologies have improved considerably, such inconsistencies in performance at field level are prevalent and significantly hamper large-scale adoption of bio-inoculants in field agriculture. Improved efficiency and continued accomplishment of rhizosphere inoculants to increase soil P availability will necessitate suitable strain identification, preparation of effective formulation and efficient agronomic delivery systems to ensure survival of inoculants.

Conclusion

Microorganisms play an important role in the cycling of P in soil–plant systems. However, this activity requires to be supplemented with crop management studies to consider their impact on P uptake by plants. This should be further aided by studies on ecological stability of the inoculants and competition from native soil microbial communities. An extensive understanding of the rhizosphere ecology, multi-trophic interactions and molecular processes associated with the augmentation of P-availability in 'responsive' soils will help in the selection and management of inoculants across diverse cropping systems, with consistently better performance. The efficiency of P-solubilizing microorganisms depends also on their exacting potentials in the soil environments and capacities to compete, colonize, survive and proliferate in the rhizosphere. Molecular tools and DNA-based diagnostic tools provide new approaches for identifying selective functional groups of soil bacteria. Application of such techniques for species- and strain-specific identification

will allow increased understanding of the interaction of inoculants with roots of crop plants (i.e. rhizosphere competence) and other rhizosphere microbiota as well as the influence of crop management practices on their continued existence. Comparative genomic, transcriptomic and proteomic characterization of microbial genotypes with and without P-solubilizing capabilities and analyses of gene expression under exacting conditions requiring P solubilization for growth have potential to identify novel isolates, their functioning and ultimately their use under field condition in an ecologically more sustainable manner[12,14].

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