Review paper

Perspectives of phosphate solubilizing microbes for plant growth promotion, especially rice - A Review

ABSTRACT

Phosphorus (P) is the second (to nitrogen) important macronutrient, a constituent of the essential macromolecules like DNA, RNA, ATP, phospholipids etc., major repository of chemical energy and indispensable at all growth stages of plants including rice. The rice crop requires around 6.4 kg P_2O_5 (2.8 kg P) per ton of grain yield and at neutral pH, P availability is optimum. Phosphate solubilizing microbes *viz*. *Bacillus*, *Pseudomonas*, *Azotobacter*, *Aspergillus* spp. recycle the nutrients like N, P, C, K, S, Fe etc. and promote plant growth and development. Different endophytes like *Rhizobium*, *Azospirillum*, *Pseudomonas* spp., epiphytes like *Rhizobium*, *Pantoea* spp., rhizospheric organisms like *Bacillus*, *Pseudomonas*, *Erwinia* spp. and entomopathogens viz. *Beauveria*, *Metarhizium, Nomuraea* spp. mineralize insoluble P for P accessiblity to plants. Soil enzymes also solubilize organic phosphates to available forms. Oxidation of glucose to gluconic acid, production of organic and inorganic acids, ammonia, H_2S, etc. are major mechanisms for acid production and P mineralization by microbes. So, phosphate solubilizing microbes would be important biofertilizers as they promote plant growth, improve soil health and protect plants from different pathogens without affecting the environment. This has led to formulation and commercialization of several P-solubilizing microbial biofertilizers.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal food which nourishes about 50, 80 and 85% world, Asian and Indian population, respectively (Zeigler and Barclay, 2008). Global harvest area of rice is second only to wheat but provides more available energy than other cereal food crops. The rice plant requires diverse nutrients whose mobility sequence is P > N > S > Mg > K > Ca (Krishna, 2010). The N, P and S are important components of protein and have higher mobility rates, absorbed rapidly during the

active vegetative growth and subsequently translocated to the grain, while the other components (e.g. silicon) have relatively lower mobility and are continuously absorbed till senescence.

Phosphorus (P) is available in water, soil and sediments (Vance *et al*. 2003), constitutes about 0.05% fraction of soil, out of which only 0.1% is available to plants. P is the second essential element (but N) for plant growth and development, makes up to about 0.2% of plant dry weight, a constituent of DNA, RNA, ATP, phospholipids and reserved chemical energy, and therefore, would be a main limiting factor for plant production, especially agricultural crops. Phosphorus deficiency effects opening of the stomata which may raise up to about 10% temperature inside the plants. Plants acquire P as phosphate anions (PO_4^{3-}) from soil which are extremely reactive and immobilized as Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+} etc. phosphates depending on soil properties which reduces use efficiency of applied phosphatic fertilizers also (Rodriguez and Fraga, 1999; Vassilev and Vassileva, 2003). Hence, regular application of soluble inorganic P (chemical fertilizers) is vital for crop production. But excessive or unmanaged P application harms the environment, for example, eutrophication and hypoxia of lakes and marine estuaries, soil degradation and loss of biodiversity which warrants use of eco-friendly fertilizers like the biofertilizers.

In soil, P may exist in 3 pools i.e. solution P, active P and fixed P (Busman *et al*. 2009). The soluble P pool is negligible comprising of orthophosphates (absorbed by plants) and organic P. As plants take up soluble phosphate, portions of the main available P forms viz. active and solid P pools are released. The fixed P forms are either insoluble inorganic phosphates and/or organic compounds that are resistant to mineralization by microorganisms. Many factors influence soil phosphorus content: (1) type of parent material from which the soil is derived, (2) extent of weathering, (3) the climatic conditions (4) erosion, crop removal and P fertilization (Osman, 1999).

The rice crop removes 0.35 kg P_2O_5/50 kg rice/hectare. For upland crops, P availability is optimum at a pH range of 6.0 to 6.5 of the soil. In acid soils (pH <6.0), P mobilization to the plants is reduced as it is coupled as iron (Fe^{3+}) and aluminum (Al^{3+}) compounds (FePO4, $AlPO_4$). At >6.5 soil pH, P could not be easily mineralized as it forms calcium and magnesium complexes ($Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$) and therefore, P uptake is limited in alkaline soils. Flooding rice soils moderates the pH towards neutrality which promotes its availability (Morales *et al*. 2014) (Fig. 1).

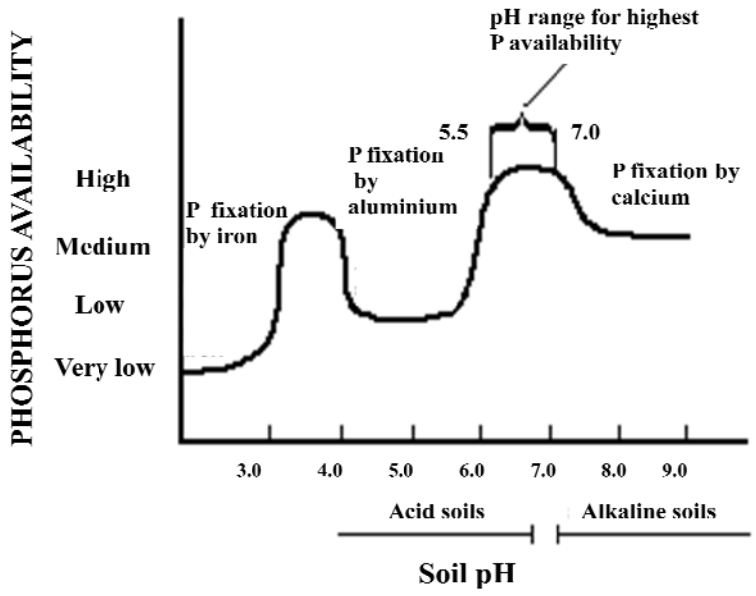

Fig. 1. Relation of available P and soil pH (Bushman *et al*. 2002)

Rice is grown in both upland and lowland ecologies. Lowland rice is grown under stagnant and partial submerged conditions during establishment and/or the vegetative and early reproductive crop growth stages. Upland rice is grown in soil that may be water saturated but never submerged during the crop growing season. P deficiency is more common in upland ecology, because the redox potential and pH of soil alter as PO_4^{2-} is released from poorly soluble or insoluble P-complexes of acid and alkaline soils after flooding. Some of the available P becomes unavailable as it binds again and precipitates as Fe^{3+} compounds after long-term flooding or seasonal drainage of a rice paddy (Kirk, 2004).

Immobilization is the tying-up of plant-available P by soil minerals and microbes that use phosphorus for their own nutritional needs. Microbes may compete with plants for P, if the decomposing organic materials are high in carbon, and low in nitrogen and phosphorus. Mineralization and immobilization occur simultaneously in soil. If the P content of the organic material is high enough to fulfill the requirements of the microbial population, then mineralization will be the dominant process (Espinoza, 2005).

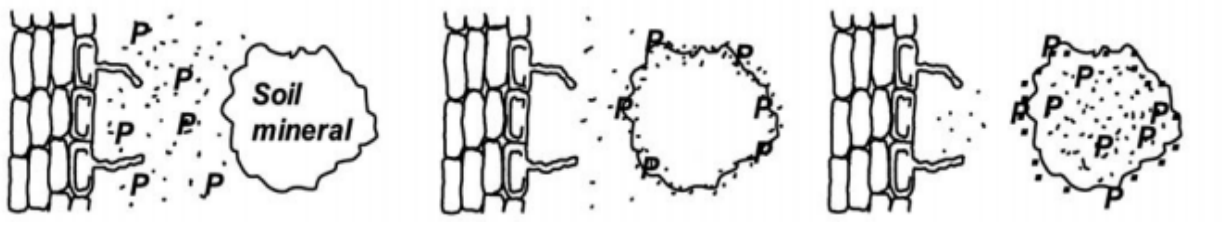

Fig. 2. Diagramatic presentation of binding of phosphorus (phosphates) with soil minerals. A) Large amount of P is available for root uptake immediately after fertilizer application. B) P in solution binds rapidly to the surface of soil minerals. Roots may still use this P. C) Most of the bound P becomes part of the structure of the mineral and significantly reduce availability to plants (Espinoza, 2005).

ROLE OF P IN RICE

P is required for energy storage and metabolic activities within the plant. It maintains membrane integrity, promotes tillering, root development, early flowering, ripening (especially at low temperature), increases straw strength and disease resistance and is particularly important in early growth stages of rice (Kimani *et al*. 2013). For optimum plant nutrition, the rice crop takes up around 6.4 kg P_2O_5 (2.8 kg P) per ton of grain yield (4.4 kg P_2O_5 in grain and 2.0 kg P_2O_5 in straw). P deficiencies resulted in stunted growth, dirty-dark green and erect leaves, lesser tillering and decreased root mass. Tillering in rice is an important agronomic characteristic for panicle formation per unit land area and reduction in yield is due to reduced tillering in P poor soil (Badshah *et al*. 2014).

PHOSPHATE-SOLUBILIZING MICROBES

Application of phosphatic fertilizers to overcome P deficiency is expensive and its overuse causes environmental hazards like eutrophication, hypoxia of lakes, etc. The situation has led to search environment-friendly and economically feasible alternate strategies to improve crop production in P-deprived soils. Phosphate solubilizing microorganisms (PSM) (Table 1) participate in bio-geochemical P cycling in natural and agricultural ecosystems to make it available for the plants, substitute (to a certain extent) chemical phosphatic fertilizers and therefore, effective ones would be competent biofertilizers which would help in rock phosphate utilization for crop production. They transform the insoluble P to soluble forms viz. HPO_4^- and $H_2PO_4^-$ by acidification, chelation, ion exchange reactions etc. Application of these microbes around the plants, in soil and with insoluble rock fertilizers releases phosphorus,

promotes plant growth, improves soil health and protects plants from pathogens without causing pollution of environment. Therefore, application of phosphate-solubilizing microbes with P-rock fertilizers would be a cost-effective and environmentally healthy and promising approach (Chang and Yang, 2009).

Table 1. List of some phosphate solubilizing microbes

Habitat	**Type of organism**	**Organisms**
Endophytic region	Bacteria	*Pseudomons*, *Bacillus* and *Klebsiella* spp., *Azorhizobium caulinodans*, *Rhizobium leguminosarum*, *Herbaspirillium seropedicae*
	Fungi	*Penicillium*, *Mortierella* spp.
	Actinomycetes	*Streptomyces*, *Micromonospora*, *Actinopolyspora*, *Saccharopolyspora*, *Nocardia* spp.
Rhizoplane	Bacteria	*Acinetobacter*, *Klebsiella* spp.
	Fungi	*Fusarium*, *Trichoderma* spp.
	Actinomycetes	*Streptomyces*, *Nocardia* spp.
Phyllosphere	Bacteria	*Rhizobium* sp.
	Fungi	*Aspergillus*, *Penicillium*, *Alternaria* spp.
	Actinomycetes	*Streptomyces* sp.
Rhizosphere	Bacteria	*Pseudomonas*, *Bacillus*, *Enterobacter*, *Arthrobacter*, *Micrococcus*, *Erwinia*, *Serratia*, *Rahnella*, *Synechococcus* spp.
	Fungi	*Aspergillus*, *Paecilomyces*, *Penicillium* spp.
	Actinomycetes	*Streptomyces* sp.

PLANT GROWTH PROMOTING MICROBES

The diverse microbial communities of any habitat maintain important functional network of vital processes of the habitats, are functionally redundant and essential to maintain the functioning of ecosystem (Bardgett and Shine, 1999). A considerable number of bacterial species such as *Alcaligenes,*

Acinetobacter, Arthrobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Paenibacillus, Pseudomonas, Rhizobium, *Serratia* spp. etc. (Table 1) are beneficial for plant growth and known as plant growth promoting bacteria (PGPB). Similarly, the naturally occurring rhizospheric soil bacteria i.e. the plant growth promoting rhizobacteria (PGPR), aggressively colonize plant roots and help in plant growth promotion. They increase nitrogen fixation in legumes and promote free-living nitrogen-fixing bacteria, enhance nutrient availability, such as, phosphorus, sulphur, iron and copper, produce plant growth regulators (PGR), enhance other beneficial bacteria or fungi, control fungal and bacterial diseases, and insect pests (Saharan and Nehra, 2011; Sharma *et al*. 2011).

Microbial exopolysaccharides (EPSs) are mainly polymers of carbohydrates (homo- or heteropolysaccharides) excreted by certain bacteria and fungi outside of the cell walls. *Enterobacter* spp. (EnHy-401, 402), *Arthrobacter* sp. (ArHy-505) and *Azotobacter* sp. (AzHy-510) produced a large amount of EPS and had strong ability for P-solubilization (Sharma *et al*. 2013). Under the same cultural conditions, *Arthrobacter* sp. could solubilize 111.7 mg P/l as the pH of culture medium lowered from 7.0 to 4.5, whereas, *Enterobacter* sp. could solubilize 632.6 mg P/l when pH decreased from 7.0 to 4.3 due to production of large amount of exopolysaccharide (Marra *et al*. 2015). Islam *et al*. (2007) showed that colonization of inoculated phosphate solubilizing bacteria (PSB) on root surface of rice seedlings depends on the rice varieties, rhizosphere environment, and extracellular polysaccharide production by bacteria. It was recorded that *Bacillus* sp. PSB16 improved plant growth in association with aerobic rice (Panhwar *et al*. 2011). Use of PSB biofertilizer increased 1 to 11% in grain yield and 6 to 8% in grain phosphorus uptake than control (Vahed *et al*. 2012) and the PSB population was more in rhizosphere and endosphere compared to non-rhizospheric soil. The rice associated PSB strains solubilized P (PSB9 strain solubilized 69.58% P), and produced organic acids, enzymes, IAA, siderophore and was antagonistic towards the pathogen *R. solani*. These beneficial characteristics would be considered as potential biofertilizer properties of PSB for aerobic rice production (Panhwar *et al*. 2012).

Like the PSB, four fungi (*Gliocladium virens*, *Trichoderma virens*, *T. harzianum* and *Aspergillus niger*) also significantly increased root length, shoot height and fresh weight of rice seedlings (Mishra and Sinha, 2000). *T. harzianum* substantially increased emergence, root and shoot length, fresh and dry weight of root of rice seedlings (Khan *et al*. 2005). Besides, the mycorrhizal fungi (MF) highly influenced

upland rice growth by increasing the shoot biomass (0.005-0.008g compared to 0.005g in control) and root biomass (0.001-0.004g compared to 0.001 in control) through increase of soil nutrients, such as, N and P. Inoculation of arbuscular mycorrhiza (AM) also imparted protection of upland rice in contaminated soil (Doni *et al*. 2013). Cyanobacteria is an important ecosystem component for addition of organic matter; synthesis and liberation of amino acids, vitamins and auxins; reduction of oxidizable matter content of the soil, contribution of oxygen to the submerged rhizosphere, amelioration of salinity, buffering pH, phosphate solubilization and intensification of fertilizer use efficiency in crop plants (Prasanna *et al*. 2009).

ENTOMOPATHOGENS

The entomopathogenic fungi like *Beauveria bassiana* (Balsamo), *Metarhizium anisopliae* (Metschnikoff), *Nomuraea rileyi* (Farlow, Samson), *Verticillium lecanii* (Zimmerman), *Fusarium* spp. (Schlechtendal) etc. are widely used biocides for management of agricultural pests. The polyvalent pathogens are suitable as both biopesticides and biofertilizers for plant growth promotion and crop production. Haas *et al*. 2008 reported that growth of plants is promoted by vacuolar and siderophore-mediated iron storage in fungi, and Kabaluk and Ericsson (2007) observed that *M*. *anisopliae* conidia treated corn seeds increased stand density, fresh weight and protected against wireworms. Besides, the polyvalent entomopathogenic bacteria viz. *B*. *thuringiensis* possessing plant growth promotion determinants like phosphate solubilization, ammonia, indole and siderophore production could be used to enhance plant growth also. The polyvalent natural entomofungal pathogens *B. bassiana* and *M. anisopliae* can infect >50% insects, as well as, possess plant growth promoting functions like phosphate solubilization (104.7-236.4 µg/ml), siderophore, ammonia production etc. which suggests that they can be exploited to control leaf folder (LF) in the rice field (Sahoo *et al*. 2013). The results proved that the biocides of leaf folder can be utilized both as plant growth promoting fungi and control of the insect pests for sustainable rice cultivation.

ENDOPHYTES

Endophytes do not express noticeable external sign of infection or negative effect and reside in the living tissues mostly in the intercellular spaces and vascular systems of the host plant in a variety of associations viz. symbiotic to slightly pathogenic. They promote growth of plants by phosphate

solubilization, indole acetic acid and siderophore production. They also supply some essential nutrients to plants and also control the deleterious effects of certain pathogenic organisms by triggering induced systemic resistance. Endophytes produce different secondary metabolites including antibiotics, anticancer compounds, volatile organic compounds, antifungal, antiviral, insecticidal and immunosuppressant agents, as well as, express necessary catabolic genes that degrade xenobiotic compounds or their metabolites which are accumulated or translocated in the vascular tissues of the host plant (Ryan *et al*. 2007). The key role in attachment and colonization on the roots is played by flagella of bacteria (Burdman *et al*. 2001). The endophytes enter the plant tissue primarily through the roots, aerial portions of plants, such as, flowers or stems, as well as, cotyledons. They produce a variety of substances of potential medicinal, agricultural and industrial application (Strobel *et al*. 2004) and are transmitted either vertically or horizontally (James *et al*. 2002). Recent metagenomic analyses of the endophytic bacterial communities colonizing rice roots suggested high potential of the microbes for plant growth promotion, improvement of stress resistance, biocontrol and bioremediation potential (Sessitsch *et al*. 2012).

Fig. 3. Schematic diagram of the different plant-bacterial endophyte interactions and their applications (Ryan *et al*. 2007)

The beneficial endophytic bacteria originate from the external environment, associate with the host plant and subsequently colonize in the internal tissues to improve performance of crops including the agricultural crops. Endophytes of rice would be various types of nitrogen-fixing and non-nitrogen-fixing bacteria (Table 1), which are found mainly in the roots, culms and seeds of various wild, traditional and cultivated varieties of rice (Barraquio *et al*. 1997). More than 600 plant species (rice, maize, wheat, grasses, *Phyllanthus amarus* etc.) are associated with dark and septate endophytic fungi, whose occurrence is related to abiotic factors, such as, low humidity in the environment and day length (Barrow and Aaltonen, 2001). Endophytic bacteria in root zone improve plant growth by increasing P availability (Duangpaeng *et al*. 2013) (Table 2). Endophytes may chemically protect the plants against other effectors like herbivore consumers (Clay, 1997). *Harpophora oryzae* incites induced systemic resistance (ISR) against the blast pathogen *Magnaporthe oryzae* and also protects rice from root invasion by different pathogens (Zhang *et al*. 2013). Resistance to virulent rice blast fungus and bacterial pathogen *Xanthomonas oryzae* was enhanced by inoculation of rice plants with the *Azospirillum* strain sp. B510 (Yasuda *et al*. 2009).

Table 2. Phosphate solubilization by rice endophytic bacteria of three organically grown rice

Isolate	Solubilization Index	Solubilization efficiency (%)
CHR2I02	2.7	72.3
CHR3I01	10.0	800.0
CHR4I07	3.2	120.3
BRR1I04	7.0	504.0
BRR3I01	3.9	186.1

(Duangpaeng *et al*. 2013)

The endophytes, such as *Azospirillum* spp., *Gluconobacter diazotrophicus, Burkholderia* spp. etc. of rice and other grasses have agribiotechnological importance (Mohanta *et al*. 2010). Rice endophytic bacteria belong to diverse genera viz. *Pseudomonas* sp., *Azoarcus* sp., *Burkholderia* sp., *Herbaspirillium seropedicae*, *Rhizobium leguminosarum*, *Serratia* sp., *Bradyrhizobium japonicum*, *Klebsiella* sp., *Azorhizobium caulinodans* etc. (Redman *et al*. 2011), and *Streptomyces* was the commonest endophytic actinomycetes in rice (Tian *et al*. 2004). Endophytes increase growth rate, reproductive yield, and

biomass of greenhouse grown rice plants by reducing 20–30% water consumption. Analyses of biomass change in non-stressed samples decreased root (30.84%) and shoot (26.13%) tissues under salt stress compared to that in non-stress condition. Symbiotic (SaltSym+) plants, however, showed lower biomass change in root (15.04%) and shoot (19.54%) tissues under salt stress compared to that in absence (SaltSym-) of stress. So, these plants exhibit enhanced stress tolerance via. symbiosis with Class 2 (non-clavicipitaceous) endophytes, and therefore, the symbiotic technology would be helpful in mitigating impacts of climate change on crops and sustain production (Redman *et al*. 2011).

Pantoea sp. (endophyte of rice) was reported to be a natural biofertilizer and bioprotective organism against several plant pathogens (Ruiz *et al*. 2011) which suggested that it would be suitable to improve productivity of rice. *Azospirillum* spp. can promote plant growth via secretion of plant growth hormones (IAA, GA), vitamins, solubilization of insoluble phosphates, decomposition of potassium, biological nitrogen fixation and induced resistance to some plant pathogens by secretion of antimicrobial compounds (Sev *et al*. 2016). Dual inoculation of *A*. *chroococcum* and *P*. *indica* (P solubilizing endophytes of rice) promoted growth of rice shoot and root both in growth chamber and field, as well as, improved growth and biomass of diverse hosts by better absorption of nutrients from the soil. Mean shoot length was recorded to be 102.23 cm that was more than control (100.67 cm) and mean root length was 36.64 cm which was more than control (35.21 cm). Different parameters of all the treatments were significantly ($P<0.05$) greater than the uninoculated control (Prajapati *et al*. 2008). Furthermore, the *B. amyloliquefaciens* NBRISN 13 strain had positive effect on growth of various plants, including rice, even under salt stress (NaCl 200 mM) condition through modulation of transcription of a set of 14 genes. Among these 14 genes, *NADP-Me2*, *EREBP*, *SOSI*, *BADH* and *SERK1* were up-regulated and *GIG* and *SAPK4* repressed under salt stress in hydroponic condition (Nautiyal *et al*. 2013).

Colonization of endophytes differs with host. Tracing of *Gluconacetobacter* sp. (PA12) (salt-tolerant, N_2-fixer and phosphate-solubilizer) tagged with *gus*A gene showed that its colonization intensity was more in *Porteresia coarctata* (wild rice) and *Pokkali* (salt-tolerant variety) than *Ponni* (salt-sensitive variety) (Loganathan and Nair, 2003). Mattos *et al*. (2008) observed promotion of both growth and grain yield of rice by the endophyte, *B*. *kururiensis*. There was an increase in root biomass of plants at 30 to 120 days postinoculation. Control of seedling disease and rice growth promotion by four endophytic

P. fluorescens, *P. tolaasii*, *P. veronii* and *Sphingomonas trueperi* strains were observed. The bacterial strains were evaluated in pot bioassays that reduced disease incidence by 50–73% (Adhikari *et al*. 2001). The endophytic bacteria i.e. CHR2I02, CHR3I01, CHR4I07, BRR1I04 and BRR3I01 of rice could solubilize tricalcium phosphate in Pikovskaya's agar and broth media and concentration of soluble phosphate was optimum ranging from 52.9 - 98.9 mg/l in 48 h after inoculation (Fig. 4) (Duangpaeng *et al*. 2012). Ability to invade and colonize by the *gus*-tagged *P. agglomerans* in the rice roots indicated that this bacterium was a true endophytic diazotroph, which produces IAA, can fix nitrogen to support growth of non-leguminous plants that faced low oxygen tension during prolonged submergence (Verma *et al*. 2001).

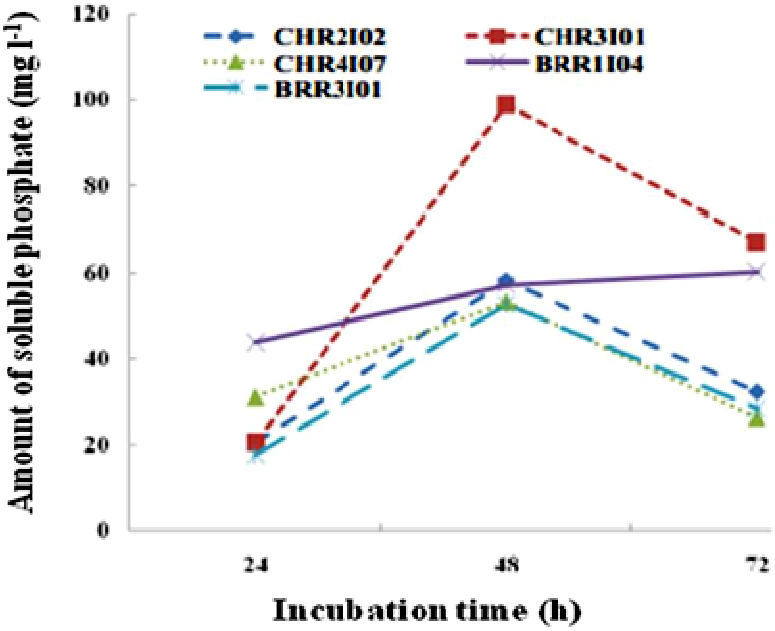

Fig. 4. Phosphate solubilization by some endophytic bacteria in Pikovskaya's broth (Duangpaeng *et al*. 2012)

EPIPHYTES

The phyllosphere is the boundary between leaves and air. The diverse microbial communities of leaves belong to different genera of bacteria, archea, filamentous fungi, yeasts, algae, and less frequently, protozoa and nematodes. Bacteria are the most numerous colonizers of leaves, often reaching to 10^6 to 10^7 cells/cm^2 of leaf (i.e. up to 10^8 cells/g). Thus, phyllosphere microbiology has much to offer to microbial ecology and have promises to contribute more effective and environment friendly plant

protection measures (Lindow and Brandl, 2003, 2006). Phyllosphere bacteria can promote plant growth, suppress or stimulate colonization and infection by plant pathogens. Colonization ecology of phylloplane and/or phyllospheric fungi is related/affected by the existing micro-environmental conditions on the leaf surfaces and their physical, chemical and phenological properties (Rasche *et al*. 2006; Mukhtar *et al*. 2010). The phyllospheric bacteria like *Rhizobium* spp. live on the leaf surface and improve growth of different plants. In rice, increase of dry weight from 17 to 47%, plant height, vegetative growth and root dry weight by 35% by the phyllophytes was observed by Santosa et al. (2003). The phyllospheric meta-proteo-genome revealed that many of the highly expressed bacterial proteins — porins, TonB-like proteins and components of ABC-type transporters are apparently involved in scavenging limited food sources on the leaf surface (Leveau, 2009).

The rhizoplane i.e. the root epidermis and outer cortex region are hot spots of plant-microbe activity where soil particles, beneficial and/or pathogenic fungal hyphae and bacteria adhere. It is the proper location to obtain antagonistic microorganisms for biocontrol of soil-borne phytopathogens. Several rhizoplane microbes like *P*. *fluorescens*, *Mycobacterium*, *Agrobacterium*, *Arthrobacter* spp. etc. supported plant growth by production of IAA and siderophores, solubilization of phosphate etc. (Singer and Munns, 2006; Ahmad *et al*. 2008). Rice seeds treated with *Acinetobacter* sp. and *Klebsiella* sp. revealed dense colonization (by the fimbriae) on the root surfaces of two-week-old seedlings. Islam *et al.* (2007) recorded that inoculation of rice seeds with phosphate solubilizing rhizoplane bacteria (Table 3, Fig. 5) may help to improve the phosphorus uptake by the rice plants.

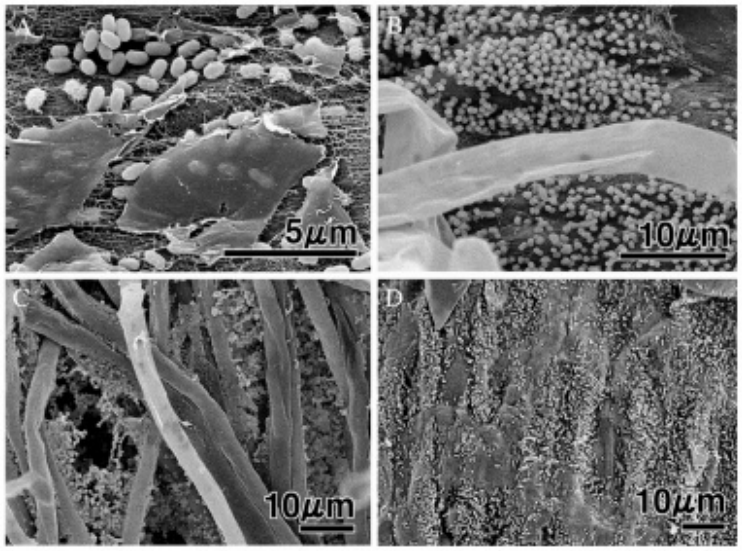

Fig. 5. Scanning electron micrographs showing colonization intensity of *Acinetobacter* sp. BR-25 (A-C) and *Klebsiella* sp. BR-15 (D) on the surface of rice (cv. BR29) root seedlings from seeds previously inoculated with bacteria (Islam *et al*. 2007).

Table 3. Calcium phosphate solubilization by some rhizoplanic bacteria

Source of rice sample	**Bacteria**	**Phosphate solubilization in NBRIP medium**	
		On agar plate (Index[a])	**In broth (µg/ml)**
Field grown rice	*Acinetobacter* sp. BR-12	4.6	387
	Klebsiella sp. BR-15	4.8	395
	Acinetobacter sp. BR-25	6.7	524
	Enterobacter sp. BR-26	3.0	206
	BR-11	1.6	40
	BR-13	2.0	94
	BR-14	1.4	68
	BR-17	1.2	2
	BR-21	1.6	73
	BR-22	1.6	11
	BR-23	1.6	8
	BR-24	2.0	86
In vitro grown rice after surface-sterilization of seeds	*Microbacterium* sp. BRS-1	2.0	97
	Pseudomonas sp. BRS-2	2.5	132

NBRIP= National Botanical Research Institute's phosphate medium; None of the isolates solubilized $AlPO_4$ or $FePO_4$. Bacterial population inoculated in liquid culture was 10^8 – 10^9 CFU/ml. [a]Solubilization index= total diameter (colony + halo zone)/colony diameter (Islam *et al*. 2007).

PHOSPHATE SOLUBILIZATION MECHANISM AND SIGNIFICANCE

Plant associated bacteria can promote plant growth directly through biological nitrogen fixation, phytohormone production, phosphate solubilization, ethylene biosynthesis inhibition of response to biotic

(induced systemic tolerance) or abiotic stresses etc., or indirectly by inducing resistance to pathogens (Bhattacharya and Jha, 2012). Plants react to P-limitation by acidification by secretion of organic acids and protons in the rhizosphere, increase growth of roots towards unexploited soil zones, increase the number of root hairs and secrete phosphatases. Mostly bacteria solubilize phosphate through production of organic acids such as gluconate, ketogluconate, acetate, lactate, oxalate, tartarate, succinate, citrate, glycolate etc. (Khan *et al*. 2009). Type of organic acid produced for P solubilization may depend upon the carbon source utilized by the microbe viz. highest P solubilization was observed when sole carbon source in the medium were glucose, sucrose or galactoses (Khan *et al*. 2009; Park *et al*. 2010).

Phosphate-solubilizing bacteria like *Bacillus*, *Enterobacter*, *Erwinia* and *Pseudomonas* spp. are the most potent ones and the organic acids, such as, gluconic acid is the major cause for release of phosphorous from mineral phosphate. Release of a range of enzymes viz. non-specific phosphatases that dephosphorylate phosphor-ester and/or phosphoanhydride bonds in organic matter, phytases that release phosphorus from phytic acid, and phosphonatases and C-P lyases that separate C-P bonds in organophophonates result in generation of soluble phosphate forms (Idriss *et al*. 2002; Rodriguez *et al*. 2006). P solubilization without acid production could be explained by release of protons accompanying respiration or ammonium assimilation (Parks *et al.* 1990). Ammonium as nitrogen source in the media effect more solubilization compared with nitrate salts (Gaur and Gaind, 1999). Production of the chelating substances (Luo *et al.* 1993), H_2S, CO_2, mineral acids, siderophores, biologically active substances like indoles, gibberllines and cytokinins are also associated with phosphate solubilization. The chelating substances cause formation of two or more coordinate bonds between an anionic or polar molecule and a cation, resulting in a ring structure complex formation which affect p-solubilization (Whitelaw, 2000).

P solubilization mainly includes efflux of low-molecular-weight organic acids (LMWOAs) and H^+ ions by roots and phosphatases capable to mobilize the organic P in soils (Fig. 2). LMWOAs compete with inorganic P (Pi) for the same sorption sites or solubilize Pi via legend-promoted mineral dissolution in acid soils dominated by Al and Fe ions. Protons released to balance the negative charge of LMWOAs may promote Pi release from soil minerals. Root efflux of citrate plays a key role in mobilization of P in aerobic rice soils. Up-regulation of high-affinity P transporters in the roots is an adaptation of plants to P deficiency. There are 26 P transporters family genes (OsPT1-26) in rice, of which first 12 are expressed

(Liu *et al*. 2011). There is a greater density of P transporter proteins per unit of root length due to over-expression of the OsPT8, which enables higher P uptake rates from the soil solution (Rose *et al*. 2012). Two main processes are involved in rhizosphere acidification: (1) Fe^{2+} is oxidized to release two protons by the radial oxygen loss (ROL) from roots; and (2) the release of more H^+ ions into the rhizosphere due to imbalance of cation/anion uptake mainly due to presence of N as NH_4^+ in reduced soil (Kirk and Saleque, 1995). ROL from roots and the subsequent oxidation of Fe^{2+} to Fe-hydroxides leads to two challenging processes like (1) mobilization of P from acid soluble P pools (presumably Ca-P minerals) as H^+ is released from the oxidation of Fe^{2+} and (2) immobilization of P as it is absorbed on Fe-hydroxides that is formed on the root surface (Fig. 6). Rather than a direct strategy to acidify the rhizosphere and solubilize P, increased ROL is the outcome of production of a low-carbon-cost root system by plants under P defficiency (Rose *et al*. 2012).

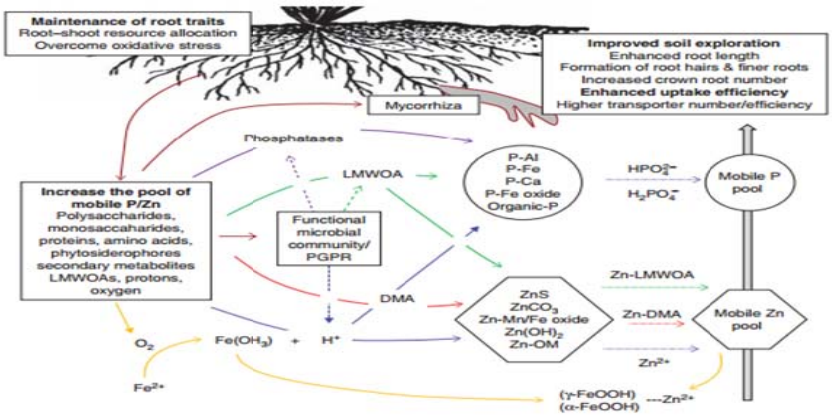

Fig. 6. Possible root induced mechanisms for P and Zn uptake from rhizosphere in rice (Rose *et al*. 2012) LMWOA- low molecular weight organic acid, DMA- deoxy mugineic acid, OM- organic matter, α-FeOOH and γ-FeOOH, iron oxyfied lepidocrocite and goethite on roots. Dashed lines with arrow indicate P/Zn uptake by roots.

MINERALIZATION OF ORGANIC P

Soil organic P mineralization plays a vital role in phosphorus cycling of farming system. Organic P may constitute 4-90% of the total soil P which can be released from organic compounds in soil by 3 groups of enzymes:

1. Nonspecific acid phosphatases (NSAPs), which carry out dephosphorylation of phosphor-ester phosphor-anhydride bonds in organic matter. Depending on the pH optima, phosphomonoesterases

enzymes are divided into acid and alkaline phosphomonoesterases. Acid phosphatases predominate in acid soils, while, alkaline phosphatases are more prevalent in neutral and alkaline soils (Sharma *et al*. 2013).

2. Phytases are a major constituent of organic P solubilization in soil that release P from phytic acid (primary source of inositol).

3. Phosphonatases and C-P lyases perform C-P cleavage in organophosphonates.

P mineralization potential by the action of phosphatases is possessed by almost half of the microorganisms in soil and plant roots (Mohammadi, 2012). Release of organic anions, and production of siderophores and acid phosphatase by plant roots/microbes (Yadaf and Tarafdar, 2001) or alkaline phosphatase enzymes hydrolyze the soil organic P or split P from organic residues. The major portion of extracellular soil phosphatases is derived from the microbial population (Dodor and Tabatabai, 2003). It was recorded that *E. agglomerans* was efficient hydroxyapatite and organic P solubilizer and mixed cultures of PSMs (*Bacillus*, *Streptomyces*, *Pseudomonas* spp. etc.) were most effective organic phosphate mineralizer.

PLANT GROWTH PROMOTION

To improve plant growth and health, PGPR use direct or indirect mechanisms. These mechanisms can be active simultaneously or sequentially at different stages of plant growth. They include:

1. Phosphate solubilization
2. Biological nitrogen fixation
3. Biological control of plant pathogens
4. Efficient uptake of other plant nutrients
5. Phytohormones like indole (indole-3-acetic acid and indole butyric acid) production

The phosphate solubilization effect seems to be the most important mechanism of plant growth promotion in moderate to higher fertile soils. Rhizobia are well known atmospheric nitrogen fixing symbiont of legumes and are potent to be used as PGPR of non-legumes also (Antoun *et al.* 1998). Biological control of plant pathogens and harmful microbes are mediated through production of antibiotics, lytic enzymes, hydrogen cyanide and siderophores. Activation of induced systemic resistance (ISR) against many pathogens, insect and nematodes is also an indirect mechanism of PGPR action

(Ramamoorthy *et al.* 2001; Zehnder *et al*. 2001). They help in competition for nutrients and space which extensively improve plant health and promote growth as evidenced by increase in seedling emergence, vigour and yield (Antoun and Kloepper, 2001). Some PGPR produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses ACC, the immediate precursor of ethylene in plants (Maheshwari, 2010) which stimulate seedlings root length by lowering ethylene concentration (Glick *et al*. 1998). Phosphorus supply is the requirement for higher yield potential of modern rice varieties, as it is crucial for energy storage and transfer within cells, speed up of root development, facilitation of N uptake and higher grain protein yield (Kimani *et al*. 2013). Both *Enterobacter* sp. strains REICA-142 and REICA-082 revealed plant-growth-promoting (PGP) properties such as N_2 fixation, inorganic phosphate solubilization and ACC deaminase production (Hardoim *et al*. 2012). *Rhizobium* may be involved in PGP activities like mobilization and efficient uptake of nutrient (Biswas *et al*. 2000a,b), enhancement in stress resistance (Mayak *et al.* 2004), solubilization of insoluble phosphates (Alikhani *et al*. 2006), ISR (Tuzun and Kloepper, 1994), production of phytohormones (Dakora, 2003), vitamins (Dobbelaere *et al*. 2003) and siderophores (Neiland and Leong, 1986). Hajiboland *et al*. (2009) stated that mycorrhizal colonization on rice significantly contributed to P and K uptake in rice plants. Yeasmin *et al*. (2007) observed that mycorrhizal enrichment and colonization on upland rice improved the soil nutrients such as N and P, as well as, growth of plants. Soil nutrients were increased (nitrogen-0.03 times and phosphorus 8 times compared to sterile soil). *P. aeruginosa* showed plant growth promoting activity and induced systemic resistance in rice against *R. solani* G5 through elevated levels of salicylic acid in host. *P. aeruginosa* pretreated rice plants produced increased levels of peroxidases with antifungal activities against *R. solani*, *P. oryzae* and *H. oryzae* (Saikia *et al*. 2006). Furthermore, inoculation of phosphate solubilizing bacteria was found to increase P uptake and yield of rice. Bacteria inoculated plants (1.98 g, 2.02 g, 2.06 g and 2.02 g respectively) had more 100 grain weight than the uninoculated control (1.97 g) (Mwashasha *et al*. 2016).

GENETICS

The organic acids, especially gluconic acid, are main factors of P mineralization. Glucose is converted to gluconic acid in presence of the enzyme (quinoprotein) glucose dehydrogenase (GDH) by direct oxidation (DO) pathway. GDH is a member of the largest group of quinoproteins, that uses the

redox cofactor 2, 7, 9- tricarboxyl-1H-pyrrolo [2, 3-f] quinoline-4, 5-dione (PQQ) and requires the metal ions such as Ca^{2+} (or Mg^{2+} *in vitro*). Gluconic acid is oxidized by gluconate dehydrogenase to 2-keto gluconic acid, which is further oxidized to 2, 5- di-keto gluconic acid (Goldstein *et al*. 2003; Krishnaraj and Dahale, 2014). Acidification of the surrounding medium occurs as the products of oxidation are directly released into the extracellular space. Oxidation generates protons which contribute directly to the trans-membrane proton motive force (PMF) resulting in uptake of exogenous amino acids and other compounds. The acidic protons dissolve the calcium phosphate complexes to soluble forms.

Genes involved in the DO pathway have been cloned from a number of bacteria. The *gdh* has been cloned and characterized from *A*. *calcoaceticus* and *E*. *coli*. The *gabY* effects expression and regulation of the DO pathway in *P*. *cepacia* and may act as a functional *Mps* gene *in vivo* (Babu-Khan *et al*. 1995; Tripura *et al*. 2005). Gene clusters encoding the enzymes to synthesize pyrroloquinoline quinone (PQQ) are cloned from *K*. *pneumoniae* and *Rahnella aquatilis* and consist of six open reading frames (*pqqA*, *B*, *C*, *D*, *E*, *F*) (Kim *et al*. 2003). Goldstein and Liu (1987) cloned a gene from *E*. *herbicola* that is involved in mineral phosphate solubilization which was revealed by screening the antibiotic resistant recombinants in a medium containing hydroxyapatite as the source of P. Expression of this gene allowed production of gluconic acid and mineral phosphate solubilization activity in *E*. *coli* HB101. Sequence analysis of this gene (Liu *et al*. 1992) suggested its probable involvement in the synthesis of the enzyme PQQ synthase, which directs the synthesis of PQQ, a co-factor necessary for the holoenzyme glucose dehydrogenase (GDH)-PQQ. New mechanisms involved in P solubilization were identified by assessing a genomic library of *P*. *fluorescens* B16 and pyrroloquinoline quinone (PQQ) biosynthetic genes were identified responsible for plant growth promotion (Choi *et al*. 2008).

For the bacteria whose genes express in *E*. *coli*, it is possible to use *E*. *coli* plasmid cloning systems to 'trap' PQQGDH genes from highly effective mineral phosphate solubilization (MPS) bacteria via functional complementation. *E*. *coli* K12 and derivatives such as DH5α constitutively synthesize *apoGDH* but do not synthesize PQQ. Therefore, screening genomic libraries of MPS bacteria for gluconic acid production traps PQQ biosynthesis genes (Liu *et al*. 1992).

Plants have come up with several strategies for coping with P deficiency. The genes up-regulated by P re-supply is important for P acquisition by P-deficient plants (Wasaki *et al*. 2006). One adaptation of

plants to P deficiency is the up-regulation of high-affinity P transporters in the roots. Manipulation of these transporters at the molecular level may improve tolerance of plants to low-P soils.

CONCLUSION

It is evident from the review that phosphate solubilizing rhizospheric, and ecto- and endophytic microbes are potent to improve plant growth and soil health through their PGP functions like biogeochemical cycling of C, N, P, K, Mg, Zn, etc. making iron availability through siderophore binding, production of extracellular enzymes and exo-polysaccharides. As the plant growth promoting endophytes live in secured niches, with limited competition, they would more effectively support the plant host. Selection of efficient phosphate metabolizing microbes would reduce phosphatic rock import and foreign exchange. The effective phosphate solubilizing genes can be used to develop self sufficient transgenic rice for P utilization or transgenic microbes can be developed by pyramiding several P-solubilizing/other PGP genes. The efficient wild or transgenic endo-colonizers would evade transgenic development. The polyvalent PGP microbes possessing biocidal functions can be formulated as biofertilizers and commercialized to sustain/improve rice production which would reduce use of hazardous chemical fertilizers and chemicides and protect environmental and biological health.

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