UNDER PEER REVIEW

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. Rice is the staple food of about 50% world and 85% Indian population which requires enhancement of production to meet the world food demand. Most agricultural soils are P deficient and therefore, necessitate continuous phosphatic fertilizer application to sustain crop production. But excess chemical P-fertilizer application bears the risk of environmental and biological health hazards. The situation has led to search for environment-friendly and economically feasible alternate strategy like the biofertilizer application to improve crop production in P deficient soils. Phosphate solubilizing microorganisms *viz*. *Bacillus*, *Pseudomonas*, *Azotobacter*, *Aspergillus* spp. etc. can mineralize insoluble P, increase P availability and substitute (at least partially) the chemical P fertilizers. Therefore, efficient biofertilizer application would promote plant growth, improve soil health and protect plants from different pathogens without affecting the environment which 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. 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.

UNDER PEER REVIEW

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).

UNDER PEER REVIEW

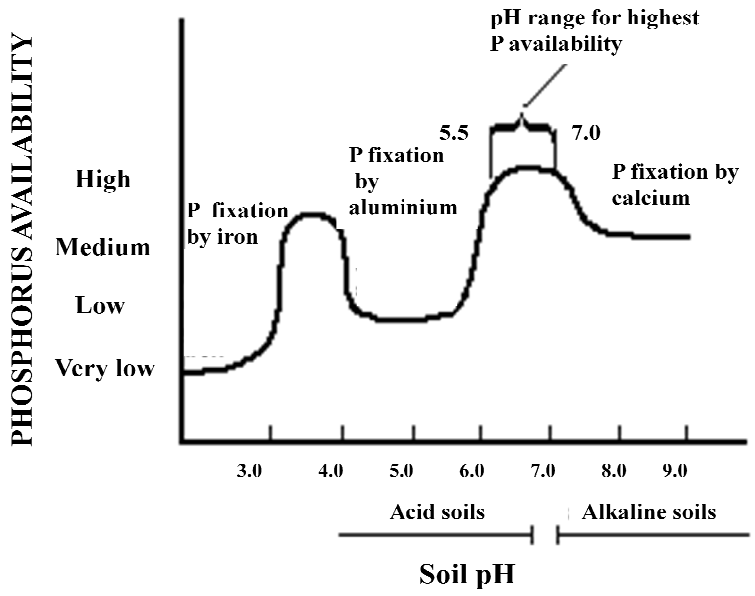

Fig. 1. Relation of available P and soil pH (Bushman et al. 2002)

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).

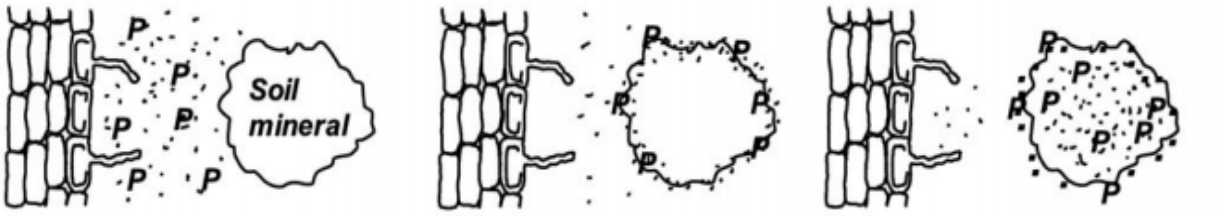

Fig. 2. Diagramatic presentation of binding of phosphorus (phosphates) with soil minerals. A) Large amount of P is available for root uptake immediately after fertilizer application. B) P in solution binds rapidly to the surface of soil minerals. Roots may still use this P. C) Most of the bound P becomes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

UNDER PEER REVIEW

ROLE OF P IN RICE

P maintains membrane integrity, promotes tillering, root development, early flowering, ripening (especially at low temperature), increases straw strength and disease resistance. It is particularly important in early growth stages of rice (Kimani et al. 2013) and 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. 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

Endophytes	Bacteria	*Pseudomons*, *Bacillus* and *Klebsiella* spp., *Azorhizobium caulinodans*, *Rhizobium leguminosarum*, *Herbaspirillium seropedicae*
	Fungi	*Penicillium*, *Mortierella* spp.
	Actinomycetes	*Streptomyces*, *Micromonospora*, *Actinopolyspora*, *Saccharopolyspora*, *Nocardia* spp.

UNDER PEER REVIEW

Rhizoplane organisms	Bacteria	*Acinetobacter*, *Klebsiella* spp.
	Fungi	*Fusarium*, *Trichoderma* spp.
	Actinomycetes	*Streptomyces*, *Nocardia* spp.
Phyllospheric organisms	Bacteria	*Rhizobium* sp.
	Fungi	*Aspergillus*, *Penicillium*, *Alternaria* spp.
	Actinomycetes	*Streptomyces* sp.
PGPR	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). 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

UNDER PEER REVIEW

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* (Panhwar et al. 2012). 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

Higher level (>50%) of infectivity of the natural entomofungal pathogens *B. bassiana* and *M. anisopliae* envisaged that they can be exploited either alone or in combination with other pathogens to control leaf folder (LF) in the rice field. The effective pathogens of leaf folder possessed polyvalent plant growth promoting functions like phosphate solubilization (104.7-236.4 µg/ml), siderophore, ammonia production etc. (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 (Ryan et al. 2007). The key role in attachment and

UNDER PEER REVIEW

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).

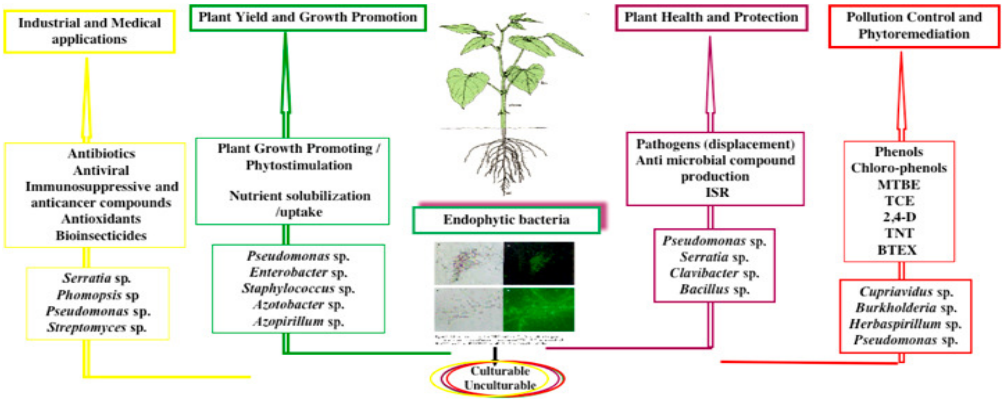

Fig. 3. Schematic diagram of the different plant-bacterial endophyte interactions and their applications (Ryan et al. 2007)

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

UNDER PEER REVIEW

Xanthomonas oryzae was enhanced by inoculation of rice plants with the *Azospirillum* strain sp. B510 (Yasuda et al. 2009).

Mutualistic 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 showed a decrease in root (30.84%) and shoot (26.13%) tissues under salt stress compared to that in absence of stress. 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.

UNDER PEER REVIEW

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).

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)

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).

UNDER PEER REVIEW

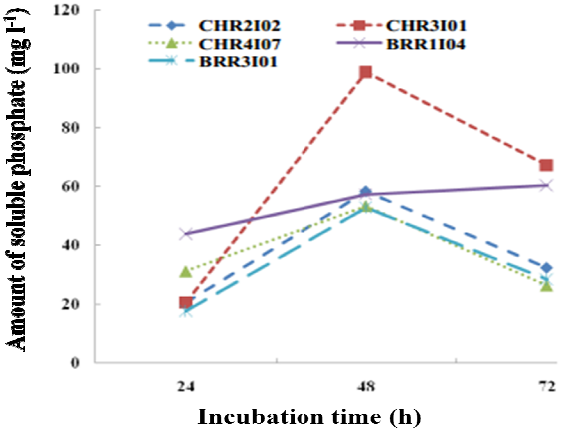

Fig. 4. Phosphate solubilization by some endophytic bacteria in Pikovskaya's broth (Duangpaeng et al. 2012)

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

UNDER PEER REVIEW

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.

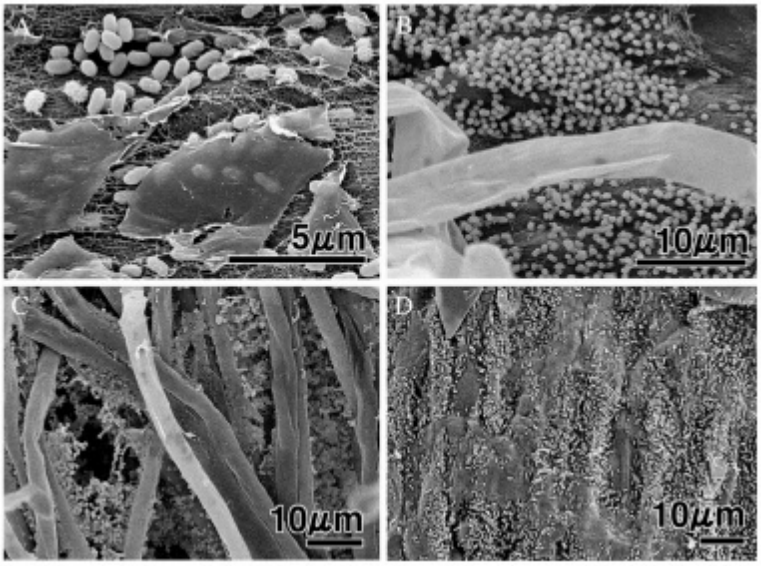

Fig. 5. Scanning electron micrographs showing colonization intensity of *Acinetobacter* sp. BR-25 (A-C) and *Klebsiella* sp. BR-15 (D) on the surface of rice (cv. BR29) root seedlings from seeds previously inoculated with bacteria (Islam et al. 2007).

Table 3. Calcium phosphate solubilization by some rhizoplanic bacteria

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

UNDER PEER REVIEW

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

UNDER PEER REVIEW

239 that dephosphorylate phosphor-ester and/or phosphoanhydride bonds in organic matter, phytases that
240 release phosphorus from phytic acid, and phosphonatases and C-P lyases that separate C-P bonds in
241 organophophonates result in generation of soluble phosphate forms (Idriss et al. 2002; Rodriguez et al.
242 2006). P solubilization without acid production could be explained by release of protons accompanying
243 respiration or ammonium assimilation (Parks et al. 1990). Ammonium as nitrogen source in the media
244 effect more solubilization compared with nitrate salts (Gaur and Gaind 1999). Production of the chelating
245 substances (Luo et al. 1993), H_2S, CO_2, mineral acids, siderophores, biologically active substances like
246 indoles, gibberllines and cytokinins are also associated with phosphate solubilization. The chelating
247 substances cause formation of two or more coordinate bonds between an anionic or polar molecule and a
248 cation, resulting in a ring structure complex formation which affect p-solubilization (Whitelaw 2000).

249 P solubilization mainly includes efflux of low-molecular-weight organic acids (LMWOAs) and H^+
250 ions by roots and phosphatases capable to mobilize the organic P in soils (Fig. 2). LMWOAs compete
251 with inorganic P (Pi) for the same sorption sites or solubilize Pi via legend-promoted mineral dissolution in
252 acid soils dominated by Al and Fe ions. Protons released to balance the negative charge of LMWOAs
253 may promote Pi release from soil minerals. Root efflux of citrate plays a key role in mobilization of P in
254 aerobic rice soils. Up-regulation of high-affinity P transporters in the roots is an adaptation of plants to P
255 deficiency. There are 26 P transporters family genes (OsPT1-26) in rice, of which first 12 are expressed
256 (Liu et al. 2011). There is a greater density of P transporter proteins per unit of root length due to over-
257 expression of the OsPT8, which enables higher P uptake rates from the soil solution (Rose et al. 2012).
258 Two main processes are involved in rhizosphere acidification: (1) Fe^{2+} is oxidized to release two protons
259 by the radial oxygen loss (ROL) from roots; and (2) the release of more H^+ ions into the rhizosphere due
260 to imbalance of cation/anion uptake mainly due to presence of N as NH_4^+ in reduced soil (Kirk and
261 Saleque 1995). ROL from roots and the subsequent oxidation of Fe^{2+} to Fe-hydroxides leads to two
262 challenging processes like (1) mobilization of P from acid soluble P pools (presumably Ca-P minerals) as
263 H^+ is released from the oxidation of Fe^{2+} and (2) immobilization of P as it is absorbed on Fe-hydroxides
264 that is formed on the root surface (Fig. 6). Rather than a direct strategy to acidify the rhizosphere and
265 solubilize P, increased ROL is the outcome of production of a low-carbon-cost root system by plants
266 under P defficiency (Rose et al. 2012).

UNDER PEER REVIEW

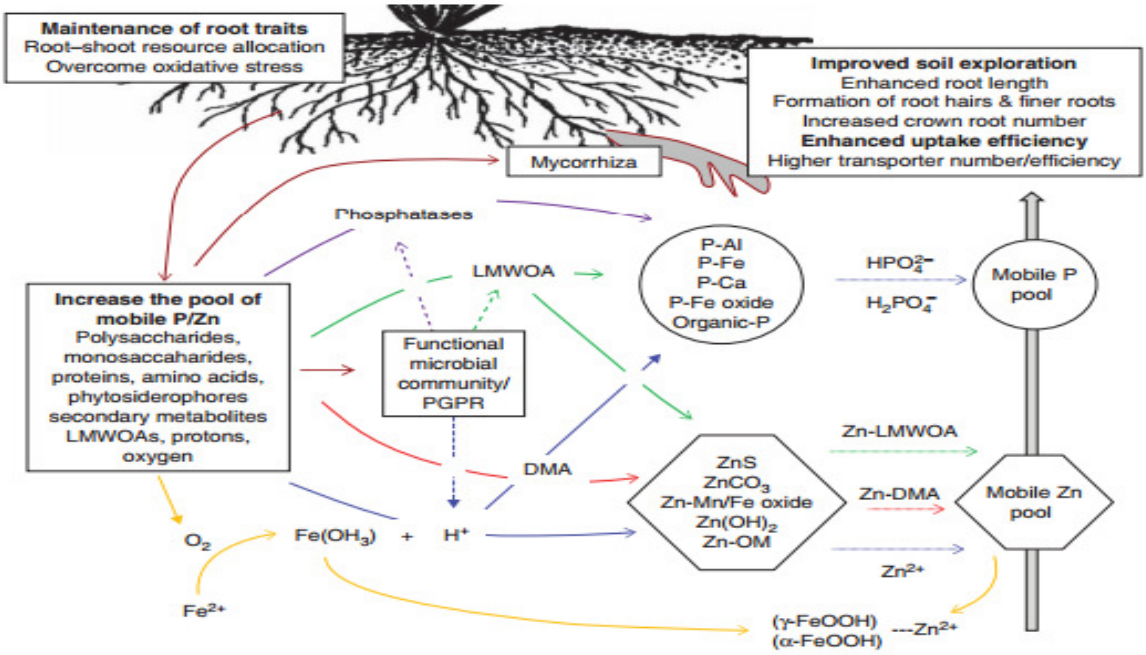

Fig. 6. Possible root induced mechanisms for P and Zn uptake from rhizosphere in rice (Rose et al. 2012) LMWOA- low molecular weight organic acid, DMA- deoxy mugineic acid, OM- organic matter, α-FeOOH and γ-FeOOH, iron oxyfied lepidocrocite and goethite on roots. Dashed lines with arrow indicate P/Zn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

UNDER PEER REVIEW

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

UNDER PEER REVIEW

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

UNDER PEER REVIEW

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.

UNDER PEER REVIEW

CONCLUSION

Appropriate investigation of rice growth promoting bacteria can be helpful for agricultural applications, higher production, reduction of input costs, and negative environmental impact due to use of fertilizers. Rice is a cash crop that earns significant foreign exchange. But the resource poor farmers cannot meet the expense of costly chemical fertilizers and pesticides to achieve the potential. Hence, there is a need for an alternative which can fulfill crop production and protection requirements on cost-effective and sustainable basis (Husssain et al. 2009). Phosphate-solubilizing bacteria perform significant function in plant nutrition through increase in P uptake by the plant, and their use as PGPR is a main contribution to biofertilization of agricultural crops. Further investigation is needed to improve performance and use of the phosphate-solubilizing bacteria as inoculants. Genetic manipulation of phosphate-solubilizing bacteria to improve their phosphate-solubilizing capabilities and/or the introduction of this trait in strains with other plant growth promoting effects is important. Use of the PSBs as bioinoculants would increase available P in soil, help to minimize the P-fertilizer application, reduce environmental pollution and promote sustainable agriculture.

Endophytes are capable of solubilizing phosphate more efficiently than rhizosphere microbes as the plants provide the nutrient requirement of the bacteria, so they do not need to compete with other soil microbes. Although work has begun on these microbial resources, still much is not known. It is necessary to study the composition and dynamics of these microbes to reach a better understanding of their diversity and P uptake by plants. Therefore, development of rice varieties with high yield under low-nutrient input has become a priority.

REFERENCES

Adhikari TB, Joseph CM, Yang G, Phillips DA, Nelson LM. Evaluation of bacteria isolated from rice for plant growth promotion and biological control of seedling disease of rice. Canadian J Microbiol. 2001;47(10):916-924.

Ahmad F, Ahmad I, Khan MS. Screening of free living rhizospheric bacteria for their multiple plant growth-promoting activities. Microbiol Res. 2008;163(2):173-181.

UNDER PEER REVIEW

Alikhani HA, Saleh-Rastin N, Antoun H. Phosphate solubilization of rhizobia native to Iranian soils. Plant Soil. 2006;287:35-41.

Antoun H, Kloepper JW. Plant growth promoting rhizobacteria. In: Brenner S, Miller JH, editors. Encyclopedia of Genetics. Academic; New York. 2001;pp 1477–1480.

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on nonlegumes: effect on radishes (*Raphanus sativus* L.). Plant Soil. 1998;204:57-67.

Babu-Khan S, Yeo TC, Martin WL, Duron MR, Rogers RD, Goldstein AH. Cloning of a Mineral Phosphate-Solubilizing Gene from *Pseudomonas cepacia*. Appl Environ Microbiol. 1995;61(3):972–978.

Badshah MA, Naimei Tu, Zou Y, Ibrahim M, Wang Ke. Yield and tillering response of super hybrid rice Liangyoupeijiu to tillage and establishment methods. The Crop Journal. 2014;2:79–86.

Bardgett RD, Shine A. Linkages between litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biol Biochem. 1999;31:317–321.

Barraquio WL, Revilla L, Ladha JK. Isolation of endophytic diazotrophic bacteria from wetland rice. Plant Soil. 1997;194(1):15–24.

Barrow JR, Aaltonen RE. A method of evaluating internal colonization of *Atriplex canescens* (Pursh) Nutt. roots by dark septate fungi and how they are influenced by host physiological activity. Mycorrhiza. 2001;11(4):199-205.

Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol. 2012;28:1327–1350.

Biswas JC, Ladha JK, Dazzo FB. Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Science Society of America Journal. 2000a;64(5):1644-1650.

Biswas JC, Ladha JK, Dazzo FB, Yanni YG, Rolfe BG. Rhizobial inoculation influences seedling vigor and yield of rice. Agron J. 2000b;92(5):880-886.

Burdman S, Dulguerova G, Okon Y, Jurkevitch E. Purification of the major outer membrane protein of Azosprillum aggregation. Molecular Plant- Microbe Interaction. 2001;14(4):555- 561.

Busman L, Lamb J, Randall G, Rehm G, Schmitt M. The nature of phosphorus in soils. University of Minnesota; 2009.

UNDER PEER REVIEW

Chang CH, Yang SS. Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. Bioresour Technol. 2009;100(4):1648–1658. doi: 10.1016/j.biortech.2008.09.009.

Choi O, Kim J, Kim J, Jeong Y, Moon J, Seuk Park C, Hwang I. Pyrroloquinoline quinone is a plant growth promotion factor produced by *Pseudomonas fluorescens* B16. Plant Physiol. 2008;146(2):657–668. doi: http://dx.doi.org/10.1104/pp.107.112748.

Clay K. Fungal endophytes, hervivores and the structure of grassland communities. In Multitrophic interactions in Terrestrial Systems, A.C. Gange and V.K.Brown, Eds. Oxford, Blackwell, 1997;pp 151-169.

Dakora FD. Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. New Phytology. 2003;158(1):39-49. doi: 10.1046/j.1469-8137.2003.00725.x.

Dobbelaere S, Vanderleyden J, Okon Y. Plant growth promoting effects of diazotrophs in the rhizosphere Crit Rev Plant Sci. 2003;22:107–149.

Dodor DE, Tabatabai MA. Effect of cropping systems on phosphatases in soils. J Plant Nutr Soil Sci 2003;166:7–13.

Doni F, Al-Shorgani NKN, Tibin EMM, Abuelhassan NN, Isahak A, Zain CRCM, Yusoff WMW. Microbial Involvement in Growth of Paddy. Current Research J Biological Sciences. 2013;5(6):285-290.

Duangpaeng A, Phetcharata P, Chanthaphoa S, Okuda N. Screening of endophyte bacteria for phosphate solubilization from organic rice. Proceeding - Science and Engineering. 2013;61–66.

Gaur AC, Gaind S. Agromicrobes: Current Trends in Life Sciences, Today and Tomorrows publishers, New Delhi, India. 1999;23,151-164.

Glick BR, Penrose DM, Li J. A model for lowering plant ethylene concentration by plant growth promoting rhizobacteria. J Theor Biol. 1998;190(1):63-68.

Goldstein AH, Lester T, Brown J. Research on the metabolic engineering of the direct oxidation pathway for extraction of phosphate from ore has generated preliminary evidence for PQQ biosynthesis in *Escherichia coli* as well as a possible role for the highly conserved region of quinoprotein dehydrogenases. Biochim Biophys Acta. 2003;1647(1-2):266–271.

Goldstein AH, Liu ST. Molecular Cloning and Regulation of a Mineral Phosphate Solubilizing Gene from *Erwinia herbicola*. Nature Biotechnol. 1987;5: 72-74. doi:10.1038/nbt0187-72.

UNDER PEER REVIEW

Hajiboland R, Afiasgharzad N, Barzeghar R. Influence of arbuscular mycorrhizal fungi on uptake of Zn and P by two contrasting rice genotypes. Plant Soil Environ. 2009;55(3):93-100.

Hardoim PR, Hardoim CCP, van Overbeek LS, van Elsas JD. Dynamics of Seed-Borne Rice Endophytes on Early Plant Growth Stages. PLoS One. 2012;7(2):e30438.

Husssain MB, Mehboob I, Zahir ZA, Naveed M, Asghar HN. Potential of *Rhizobium* spp. for improving growth and yield of rice (*Oryza sativa* L.). Soil & Environ. 2009;28(1):49-55.

Idriss EE, Makarewicz O, Farouk A, Rosner K, Greiner R, Bochow H, Richter T, Borriss R. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. Microbiology. 2002;148(Pt 7):2097-109.

Islam MdT, Deora A, Hashidokoa Y, Rahmana A, Itoa T, Tahara S. Isolation and Identification of Potential Phosphate Solubilizing Bacteria from the Rhizoplane of *Oryza sativa* L. cv. BR29 of Bangladesh. Z. Naturforsch. 2007;62c,103D110.

James EK, Gyaneshwar P, Mathan N, Barraquio WL, Reddy PM, Iannetta PP, Olivares FL, Ladha JK. Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. Mol Plant Microbe Interact. 2002;15(9):894–906.

Khan AA, Sinha AP, Rathi YPS. Plant growth promoting activity of *Trichoderma herzianum* on rice seed germination. Indian J Agric Res. 2005;39(4):256-262.

Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA. Plant growth promotion by phosphate solubilizing fungi – current perspective. Arch Agron Soil Sci. 2009;56(1):73–98.

Kim CH, Han SH, Kim KY, Cho BH, Kim YH, Koo BS, Kim YC. Cloning and expression of pyrroloquinoline quinone (PQQ) genes from a phosphate-solubilizing bacterium *Enterobacter intermedium*. Curr Microbiol. 2003;47(6):457–461.

Kimani JM, Tongoona P, Derera J. Breeding dynamics of rice (*Oryza sativa*) for enhanced adaptation and grain quality. Sci Res Essays. 2013;8(27):1258-1272.

Kirk GJD. The biogeochemistry of submerged soils. Chichester, UK: John Wiley & Sons; 2004.

Krishna KR. Agroecosystems of South India: nutrient dynamics, ecology and productivity. BrownWalker Press, Boca Raton, Florida, USA; 2010. ISBN 978-1-59942-534-4

Leveau J. Life on leaves. NATURE. 2009;461(7265):741.

UNDER PEER REVIEW

Lindow SE, Brandl MT. Microbiology of the Phyllosphere. Appl Environ Microbiol. 2003;69(4):1875–1883. doi:10.1128/AEM.69.4.1875-1883.2003.

Liu ST, Lee LY, Taj CY, Hung CH, Chang YS, Wolfrang JH, Rogers R, Goldstein AH. Cloning of an *Erwinia herbicola* gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in *Escherichia coli* HB101: nucleotide sequence and probable involvement in biosynthesis of the coenzyme Pyrroloquinoline Quinone J Bacteriol. 1992;174(18):5814–5819.

Loganathan P, Nair S. Crop-specific endophytic colonization by a novel, salt-tolerant, Nitrogen fixing and phosphate-solubilizing *Gluconacetobacter* sp. from wild rice Biotechnol. Lett. 2003;25(6):497–501. doi: 10.1023/A:1022645124506.

Luo AC, Sun X, Zhang US. Species of insoluble Phosphate Solubilizing Bacteria in Rhizosphere and their effect on yield on yield and nutrient uptake of wheat crop. Plant and Soil. 1993;57:223-230.

Maheshwari DK. Plant growth and health promoting bacteria. Microbiology monograph, vol. 18. Springer. Heidelberg; 2010.

Mayak S, Tirosh T, Glick BR. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem. 2004;42(6):565-72.

Mishra DS, Sinha AP. Plant growthpromoting activity of some fungal and bacterial agents on rice seed germination and seedling growth. Trop Agric. 2000;77:188-191.

Mohammadi K. Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and Their Role in Crop Production. Resources Environment. 2012;2(1):80-85.

Mohanta S, Sharma GD, Deb B. Diversity of Endophytic Diazotrophs in Non-Leguminous Crops - A Review. Assam University Journal of Science & Technology: Biological and Environmental Sciences 2010;6(1):109-122.

Morales LA, Vázquez EV, Paz-Ferreiro J. Spatial Distribution and Temporal Variability of Ammonium-Nitrogen, Phosphorus, and Potassium in a Rice Field in Corrientes, Argentina. The Scientific World Journal. 2014;Article ID 135906:1-12

Mukhtar I, Mushtaq S, Ali A, Khokhar I. Epiphytic and endophytic phyllosphere microflora of *Cassytha filiformis* L. and its hosts. ECOPRINT. 2010;17:1-8.

UNDER PEER REVIEW

Nautiyal CS, Srivastava S, Chauhan PS, Seem K, Mishra A, Sopory SK. Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. Plant Physiol Biochem. 2013;66:1-9.

Neiland JB, Leong SA. Siderophores in relation to plant disease. Annu Rev Plant Physiol. 1986;37,187–208.

Osman A. Soil Fertility Management in Cajamarca, Peru. Centro Internacional de la Papa (CIP), Lima; 1999.

Panhwar QA, Othman R, Rahman ZA, Meon S, Ismail MR. Effect of Phosphatic Fertilizer on Root Colonization of Aerobic rice by Phosphate-Solubilizing Bacteria. International Conference on Food Engineering and Biotechnology IPCBEE. 2011;9:145-149.

Panhwar QA, Othman R, Rahman ZA, Meon S, Ismail MR. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. African J Biotechnol. 2012;11(11):2711-2719.

Park KH, Lee OM, Jung HI, Jeong JH, Jeon YD, Hwang DY, Lee CY, Son HJ. Rapid solubilization of insoluble phosphate by a novel environmental stress-tolerant *Burkholeria vietnamiensis* M6 isolated from ginseng rhizospheric soil. Appl Microbiol Biotechnol. 2010;86(3):947-955.

Parks EJ, Olson GJ, Brinckman FE, Baldi F. Characterization by high performance liquid chromatography (HPLC) of the solubilization of phosphorus in iron ore by a fungus. J Ind Microbiol Biotechnol. 1990;5:183-189.

Prajapati K, Yami KD, Singh A. Plant Growth Promotional Effect of *Azotobacter chroococcum*, *Piriformospora indica* and Vermicompost on Rice Plant. Nepal J Science and Technology. 2008;9:85-90. doi: 10.3126/njst.v9i0.3170.

Prasanna R, Nain L, Ancha R, Srikrishna J, Joshi M, Kaushik BD. Rhizosphere dynamics of inoculated cyanobacteria and their growth-promoting role in rice crop. Egyptian J Biol. 2009;11:26-36.

Ramamoorthy V, Viswanathan R, Raghuchander T, Prakasam V, Samiyappan R. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protect. 2001;20:1–11.

UNDER PEER REVIEW

Rasche F, Trondl R, Naglreiter C, Reichenauer TG, Sessitsch A. Chilling and cultivar type affect the diversity of bacterial endophytes colonizing sweet pepper (*Capsicum anuum* L.). Can J Microbiol. 2006;52(11):1036–1045.

Redman RS, Kim YO, Woodward CJDA, Greer Chris Espino L, Doty SL, Rodriguez RJ. Increased Fitness of Rice Plants to Abiotic Stress Via Habitat Adapted Symbiosis: A Strategy for Mitigating Impacts of Climate Change. PLoS ONE. 2011;6(7):e14823.

Rodríguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv. 1999;17(4-5):319–339.

Rodriguez H, Fraga R, Gonzalez T, Bashan Y. Genetics of phosphate solubilisation and its potential applications for improving plant growth-promoting bacteria. Plant Soil. 2006;287:15-21. doi: 10.1007/s11104-006-9056-9.

Rose TJ, Impa SM, Rose MT, Pariasca-Tanaka J, Mori A, Heuer S, Johnson-Beebout SE, Wissuwa M. Enhancing phosphorus and zinc acquisition efficiency in rice: a critical review of root traits and their potential utility in rice breeding. Ann Bot. 2013;112(2):331–345.

Ruiz D, Agaras B, de Werrab P, Wall LG, Valverde C. Characterization and screening of plant probiotic traits of bacteria isolated from rice seeds cultivated in Argentina. J Microbiol. 2011;49(6):902-912.

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett. 2007;278(1):1-9.

Saharan BS, Nehra V. Plant growth promoting rhizobacteria: A critical review. Life Sci Med Res. 2011; LSMR-21,1-30.

Saikia R, Kumar R, Arora DK, Gogoi DK, Azad P. *Pseudomonas aeruginosa* inducing rice resistance against *Rhizoctonia solani*: production of salicylic acid and peroxidases. Folia Microbiol (Praha). 2006;51(5):375-80.

Santosa DA, Handayanf N, Iswandi dA. Isolation and Selection o/Growth Promoting Phyllosphere Bacteria/rom Lea/o/ Rice (Oryza sativa L.) Cultivar IR·64. IIlmal Tanah dan Lingkungan. 2003;5(1):7-12.

Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Mitter B, Hauberg-Lotte L, Friedrich F, Rahalkar M, Hurek T, Sarkar A, Bodrossy L, van Overbeek L, Brar D, van Elsas JD, Reinhold-Hurek B.

UNDER PEER REVIEW

Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant Microbe Interact. 2012;25(1):28-36.

Sharma SB, Sayyed RZ, Trivedi MH Gobi TA. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*. 2013;2:587.

Singer MJ, Munns DN. Soils: An Introduction. Sixth Edition. Pearson Prentice Hall, Upper Saddle River, New Jersey; 2006.

Strobel G, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms. J Nat Prod. 2004;67(2):257–268.

Tian XL, Cao LX, Tan HM, Zeng QG, Jia YY, Han WQ, Zhou SN. Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their anti-pathogenic activities in vitro. World J Microbiol Biotechnol. 2004;20:303–309. doi: 10.1023/B:WIBI.0000023843.83692.3f.

Tripura CB, Sashidhar B, Podile AR. Transgenic mineral phosphate solubilizing bacteria for improved agricultural productivity. In Microbial Diversity Current Perspectives and Potential Applications ed. Satyanarayana, T. and Johri, B.N. New Delhi, India: I. K. International Pvt. Ltd. 2005;375-392.

Tuzun S, Kloepper JW. Induced systemic resistance by plant growth-promoting rhizobacteria. In: Improving Plant Productivity with Rhizosphere Bacteria p.104-109. In: M.H. Ryder, P.M. Stephens and G.D. Bowen (eds). CSIRO, Adelaide, South Australia, Australia; 1994.

Vahed HS, Shahinrokhsar P, Heydarnezhad F. Performance of phosphate solubilizing bacteria for improving growth and yield of rice (*Oryza Sativa* L.) in the presence of phosphorus fertilizer. Int J Agri Crop Sci. 2012;4(17):1228-1232.

Vance CP, Uhde-Stone C, Allan DL. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 2003;157(3):423–447. doi: 10.1046/j.1469-8137.2003.00695.x.

Vassilev N, Vassileva M. Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes. Appl Microbiol Biotechnol. 2003;61(5):435-440.

Verma SC, Ladha JK, Tripathi AK. Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. J Biotechnol. 2001;91(2-3):127–141.

UNDER PEER REVIEW

Wasaki J, Shinano T, Onishi K, Yonetani R, Yazaki J, Fujii F, Shimbo K, Ishikawa M, Shimatani Z, Nagata Y, Hashimoto A, Ohta T, Sato Y, Miyamoto C, Honda S, Kojima K, Sasaki T, Kishimoto N, Kikuchi S, Osaki M. Transcriptomic analysis indicates putative metabolic changes caused by manipulation of phosphorus availability in rice leaves. J Exp Bot. 2006;57(9):2049–2059.

Whitelaw Ma. Growth promotion of plants inoculated with phosphate-solubilizing fungi. Adv Agron. 2000;69:99-151.

Yadav RS Tarafdar JC. Influence of Organic and Inorganic Phosphorous Supply on the Maximum Secretion of Acid Phosphatase by Plants. Biology and Fertility of Soils. 2001;34(3):140-143.

Yasuda M, Isawa T, Shinozaki S, Minamisawa K, Nakashita H. Effects of Colonization of a Bacterial Endophyte, *Azospirillum* sp. B510, on Disease Resistance in Rice. Biosci Biotechnol Biochem. 2009;73(12):2595-2599.

Yeasmin T, Zaman,P, Rahman A, Absar N, Khanum NS. Arbuscular mycorrhizal fungus inoculum production in rice plants. Afr J Agric Res. 2007;2(9):463-467.

Zehnder GW, Murphy JF, Sikora EJ, Kloepper JW. Application of rhizobacteria for induced resistance. Eur J Plant Pathol. 2001;107(1):39–50. doi: 10.1023/A:1008732400383.

Zhang C, Feng X, Su Z, Xu X, Wang J, Liu N. Evidence for biotrophic lifestyle and biocontrol potential of dark septate endophyte *Harpophora oryzae* to rice blast disease. In: Schneider C, Leifert C, Feldmann F (Eds), Endophytes for plant protection: the state of the art, 2013;249-257.