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Mechanisms of Plant Growth Promotion by Rhizobacteria

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The principal goal of agriculture is the production of high quality, safe and affordable food for an ever-increasing population worldwide. Furthermore, agricultural growers and producers have the additional constraints of economic profitability and sustainability. Looking at the negative environmental impact of chemical fertilizers, the use of beneficial soil microorganisms for sustainable and safe agriculture has increased globally during the last couple of decades. Plant growth promoting rhizobacteria are naturally occurring soil bacteria that assertively colonize plant roots and benefit plants by providing growth factors. Stress-relieving and antagonistic rhizobacteria might be useful in formulating new inoculants with combinations of different mechanisms of action, leading to a competent use for biocontrol strategies to improve cropping systems.

Key words: PGPR, Siderophore, P-solubilization, N-fixation, ACC-deaminase.

The rhizosphere, as defined by Bowen and Rovira (1999)[1], is a tender zone of soil surrounding a plant root (about 1-3mm) where living organisms are influenced by vital root activities (root exudates and respiration) qualitatively and quantitatively. The term 'rhizobacteria' implies a group of rhizospheric bacteria competent in colonizing the root environment (Kloepper *et al*. 1991)[2]. Plant-associated bacteria can be classified into beneficial, deleterious and neutral groups on the basis of their effects on plant growth (Dobbelaere *et al.,* 2003)[3]. Beneficial free-living soil bacteria are usually referred to as plant growth-promoting rhizobacteria (PGPR, Villacieros *et al*. 2003[4]; Beneduzi *et al*. 2013[5]). *Azotobacter*, *Enterobacter*, *Bacillus*, *Burkholderia*, *Azospirillum*, *Pseudomonas*, *Acinetobacter*, *Arthrobacter*, *Alcaligenes*, *Serratia*, *Erwinia* and *Flavobacterium* are some of the common PGPRs (Bloemberg and Lugtenberg 2001)[6]. These are heterogeneous bacteria which can improve the extent or quality of plant growth direct or indirectly (Joseph *et al*. 2007)[7]. Direct promotion of growth by PGPR occurs when the rhizobacteria improve supply of nutrients, such as nitrogen and phosphorus and produce metabolites such as auxins, cytokinins and gibberellins. Indirect plant growth promotion occurs through the elimination of pathogens by the production of cyanide, siderophores and chitinases (Table 1). Beneficial effects of PGPR have been exploited in many areas including biofertilizers, microbial rhizomediation and biopesticides (Adesemoye *et al*. 2008)[8]. In addition, recently, it has also been reported that some rhizobacteria contain an enzyme ACC-deaminase which can reduce ethylene biosynthesis in plants. Thus, plants are benefited in a number of ways through direct uptake of iron, suppression of proliferation of fungal pathogens, improved N-fixation and prevention from heavy metal toxicity.

Direct growth promoting attributes

Production of growth hormones

In 1880, Charles Darwin proposed that some plant growth responses are regulated by 'a

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matter which transmits its effects from one part of the plant to another' (Kevin, 2003)[9]. Several decades later, this 'matter', termed auxin, was identified as indole-3-acetic-acid (IAA) (Spaenpan *et al*. 2007)[10]. IAA is quantitatively the most abundant type of auxin that plays a crucial role in many developmental processes in plants (Woodward and Bartel 2005)[11]. IAA is responsible for division, expansion and differentiation of plant cells and tissues and also stimulates elongation. Although plants are able to synthesize IAA themselves, the microorganisms that are the inhabitants of rhizosphere also contribute to plant's auxin pool (Arkhipova *et al.* 2005)[12]. The ability to synthesize IAA has been detected in many rhizobacteria as well as in pathogenic, symbiotic and free-living bacterial species. In order to produce IAA, the bacteria use tryptophan as precursor (Asghar *et al.* 2004)[13]. The release of L-tryptophan in root exudates may result in its conversion into IAA by rhizosphere microbes (Kravchenko *et al*. 2004)[14]. Phytopathogenic bacteria mainly use indole acetamide pathway to synthesize IAA, which has been implicated in tumour induction in plants. In contrast, acid indole pyruvate appears to be the main pathway present in PGPR (Patten and Glick 2002)[15]. Major IAA producing bacteria belong to *Aeromonas*, *Bacillus*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Pseudomonas* and *Rhizobium* genera (Swain *et al*. 2007[16]; Ahmad *et al*. 2008[17]; Hariprasad and Niranjana 2009[18]), with some exceptions (Sivasakthivelan and Saranraj 2013) [19] . IAA production was significantly higher in *Pseudomonas*, *Rhizobium* and *Azotobacter chroococcum* as compared to *Bacillus megaterium* as demonstrated by Ahmad *et al*. (2008)[17]. Inoculation with IAA producing PGPR has been used to stimulate seed germination to accelerate root growth and modify the architecture of the root system and to increase the root biomass. In case of *Azospirillum*, bacterial colonization occurred in the zone of lateral root emergence. *Azospirillum* inoculation increased the density and length of root hairs as well as the root surface area (Dobbelaereb *et al*. 2001)[20] and thereby the microbial activity. Swain *et al.* (2007) [16] reported a positive effect of *Bacillus subtilis* IAA producing strains on the edible tubercle *Dioscorea rotundata* L. in one of their studies. They applied a suspension of *B. subtilis* on the surface of the plants, which resulted in an increase in stem and root length, increased fresh weight of the stem and root, an increase in the root-stem ratio and increased numbers of sprouts as compared with non-inoculated plants. Auxin regulates the expression of different genes in *Rhizobium*-legume interactions that are involved in plant signal processing and attachment to plant roots. Moreover, changes in auxin balance in host plants are prerequisites of IAA and can promote the production of 1-aminocyclopropane-1-carboxylate (ACC), precursor of ethylene synthesis and result in inhibition of seed germination and root growth (Andrei and Belimov 2002) [21].

Microorganisms are also capable of producing cytokinins, gibberellins, ethylene (ET), or abscisic acid. Ethylene, a hormone produced in all plants, mediates several responses to developmental and environmental signals in plants. Its involvement in plant growth when excreted around the roots has also been shown (Arshad and Frankenberger 1998)[22]. Cytokinins and gibberellins are produced in the rhizosphere by several bacteria, e.g. *Azospirillum*, *Agrobacterium* and *Pseudomonas* genera (Gaudin *et al*. 1994)[23]. Cytokinins promote root formation, but a minor overproduction instead, leads to inhibition of root development, and severely deficient cytokinin mutant plants do not survive (Binns 1994) [24]. Cytokinins are believed to be the signals involved in mediation of environmental stresses from roots to shoots (Jackson 1993)[24]. Thus, PGPR can facilitate growth by altering the hormonal balance in the affected plant. It has been reported to be produced by certain rhizospheric bacteria like *Bacillus licheniformis* and *Bacillus pumilus* (Gutierrez-Manero *et al.* 2001) [26].

Phosphate solubilization

Phosphorus (P) is second only to nitrogen among mineral nutrients most commonly limiting the growth of legumes. P is an essential plant nutrient with low availability in many agricultural soils. In many soils, application of phosphatic fertilizers is a must to make up for the P lost due to the fixation of soluble phosphate by the soil constituents and phosphate run off in P-loaded soils (Vikram and Hamzehzarghani 2008)[27]. Phosphate anions are extremely reactive

and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{2+}, depending on particular properties of a soil. On the other hand, much of this P is in mineral form and is only slowly available to plants (Richardson *et al*. 2009)[28]. The role of microorganisms in solubilizing inorganic phosphates in soil and making them available to plants is well known (Bhattacharya and Jain 2000)[29]. They are known P-solubilizers and convert insoluble phosphates into soluble forms by acidification, chelation, exchange reactions and production of gluconic acid (Chen *et al*. 2006)[30], thereby releasing fixed or insoluble P in available form (Fig 1). Phospate Solubilizing Bacteria (PSB) involved in P solubilization and mineralization act principally by acid phosphatases, catalyzing the hydrolysis of phosphoric esters (Glick 2012)[31]. Another mechanism of phosphate solubilization is by phytase production, since organic P can constitute between 30 to 50% of the total P of the soil, a high proportion of which corresponds to phytate (Turner *et al*. 2003)[32]. Phytase producing rhizobacteria have been reported to belong to genera *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Serratia* and *Staphylococcus* (Shedova *et al.* 2008)[33]. Further, Jorquera *et al.* (2008) [34] isolated PSB from the rhizosphere of 5 cultivated plants (*Lolium perenne*, *Trifolium repens*, *Triticum aestivum*, *Avena sativa* and *Lupinus luteus*), which presented more than one mechanism for utilizing insoluble form of P. Moreover, all strains showed the capacity to produce P hydrolases. Recent reports have revealed that optimum P-solubilization takes place in presence of NaCl concentration from 0 to 1.25% but higher concentrations increases time of P-solubilization from 48 to 72 h (Deshwal and Kumar 2013) [35].The major limitation today, for use of these organisms is the lack of consistent effects in mobilizing P under field conditions. This is likely due to competition with the native microflora and environmental factors that either limit the population size or activity of the PGPR. Microbial biomass assimilates soluble P and prevents it from absorption or fixation (Khan and Joergesen 2009) [36].

Indirect plant growth promoting attributes

Siderophore production

Iron (Fe) is a growth requirement of virtually all living organisms. The insolubility of Fe under oxidized conditions at neutral or alkaline pH necessitates special mechanisms for iron acquisition in most organisms. Microorganisms have evolved specialized mechanisms for the assimilations of iron, including production of iron chelating compounds, known as siderophores. Siderophores are low molecular weight (500-1000 Da) compounds that are produced and utilized by bacteria and fungi. These compounds are produced in response to iron deficiency which normally occurs in neutral to alkaline soils, due to low iron solubility at elevated pH (Sharma and Johri 2003)[37]. Further, most of the catechols are derivatives of 2, 3-dihydroxy benzoic acid (DHBA) and consists of 2, 3-DHBA and one or more amino acid residues (Xie *et al*. 2006)[38]. Any factor influencing either the growth or siderophore production by PGPR would greatly influence the efficacy of that PGPR in plant growth promotion and disease suppression (Chincholkar *et al*. 2000)[39]. Siderophores have been implicated for both direct and indirect enhancement of plant growth by rhizospheric microorganisms (Neilands 1981)[40]. The iron-siderophore complex is absorbed by the plants to quench iron thirst in calcareous soil, a direct mechanism (Sharma and Johri 2003)[37], whereas chelation of soluble iron by microbial siderophores result in shift in rhizospheric communities (Bano and Musarrat 2003)[41], being an indirect mechanism. Ligand exchange reaction may also be another possible mechanism. In both Gram-negative and Gram-positive rhizobacteria, iron (Fe^{3+}) in Fe^{3+}-siderophore complex on bacterial membrane is reduced to Fe^{2+} which is further released into the cell from the siderophore via a gating mechanism linking the inner and outer membranes (Ahemad and Kibrat 2014)[42]. During this reduction process, the siderophore may be destroyed/recycled (Rajkumar *et al*. 2010)[43]. Besides microbial iron nutrition, many siderophores also play a very important role in microbial infection and the antagonism of PGPR against plant pathogen. Siderophore producing PGPR can prevent the proliferation of pathogenic microorganisms by sequestering Fe^{3+} in the area around the root (Siddiqui 2006)[44]. Fe depletion in the rhizosphere does not affect the plant, as the low Fe

concentrations occur at microsites of high microbial activity during establishment although the total concentrations are probably too low to contribute substantially to plant iron uptake. In recent years, considerable interest has been paid to rhizobacteria, which are aggressive root colonizers and produce siderophores (Roy and Chakrabartty 2000 [45]; Khandelwal *et al*. 2002 [46]; Kuffner *et al*. 2008 [47]; Ahemad and Khan 2012f [48]; Kannahi and Kowsalya 2013 [49]; Goswami *et al*. 2014 [50]). Singh *et al*. (2014) [51] tested the ability of *Pseudomonas* to grow and to produce siderophores in the presence of different carbon, nitrogen sources and pH. Maximum catechol-type siderophore production at pH=7 was obtained by ML-I (88.6 ¼g/ml) and hydroxamate by SH-IV (15.6 ¼g/ml) while growth in terms of optical density by BM-II (OD600nm 1.84). Among the carbon and nitrogen sources, glucose (0.4%) and L-Lysine and L-Arginine (0.1%) were found to increase siderophore production as well as growth.

Antifungal activity of rhizobacteria against different plant pathogenic fungi

A large body of information has been accumulated regarding antagonism between bacteria and fungi on the leaf surface, and its possible role in the biological control of pathogenic fungi (Gowdu and Balasubramanian 1988)[52]. Biological control may offer an alternative to chemicals in the control of some pathogenic fungi and also reduce environmental pollution. The first clear indication of improved plant growth and biological control of root pathogens due to seed bacterization with rhizobacteria came from the works of Burr *et al*. (1978) [53] and Kloepper *et al*. (1980) [54] who reported the plant growth promoting effects of *Pseudomonas* strains which were antagonistic to a wide range of plant pathogens *in vitro*. These

Table1. Growth promoting substances released by PGPR

PGPR	Plant Growth Promoting Traits	References
Pseudomonas putida	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization	Ahemad and Khan 2012a [103] Ahemad and Khan c[104]
Pseudomonas aeruginosa	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization	Ahemad and Khan 2012e[105]
Rhizobium sp. (pea)	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan 2012b[106]
Mesorhizobium sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan 2012d[107]
Bradyrhizobium sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides	Ahemad and Khan 2012f [48]
Pseudomonas fluorescens	IAA, siderophores	Saranraj *et al*. 2013[108]
Pseudomonas aeruginosa, *P. putida*, *P. cepacia* and *P. fluorescens*	Indole acetic acid (IAA), Hydrogen cyanide (HCN), Siderophore and Phosphorous solubilization	Deshwal and Kumar 2013[35]
Pseudomonas sp.	(IAA), ammonia (NH_3), hydrogen cyanide (HCN), siderophore, phosphate (P) solubilization, catalase	Kaur and Sharma 2013[109]
Bacillus amyloliquefaciens, *Serratia marcescens* and *B. pumilus*	IAA, Siderophore and Phosphorous solubilization	Chakraborty *et al*. 2013[110]
Pseudomonas fluorescens and *Bacillus subtilis*	IAA, Hydrogen cyanide (HCN), Siderophore and Phosphorous solubilization, catalase	Kannahi and Kowsalya 2013[49]
Pseudomonas aeruginosa	IAA, Hydrogen cyanide (HCN), Siderophore and Phosphorous solubilization, catalase, urease production	Goswami *et al*. 2014[50]
Pseudomonas fluorescens	Siderophore	Singh *et al*. 2014[51]

studies also provided the first evidence that the rhizosphere microbiota could be modified significantly with microorganisms introduced with the planting material. Rhizobacteria are ideal for use as biocontrol agents since they inhabit the rhizosphere that provides the front line defense for roots against attack by pathogens. The major mechanisms by which most PGPR exert their antagonistic effect against fungal pathogens include antibiosis, competition, parasitism, siderophore production and induction of systemic resistance (Sadfi *et al*. 2001)[55]. *Pseudomonas* spp. have been investigated as potential bioantagonists against plant pathogens due to their ability to colonize the rhizosphere and protect plants against a range of agronomically important fungal diseases (Kaur *et al*. 2007)[56]. Tiwari and Thrimurthy (2007) [57] reported that *in vitro* evaluation of *P. fluorescens* isolates from the rhizosphere of wheat, chickpea, mung, urdbean, soybean and sunflower confirmed their antagonistic ability against both *Pyricularia grisea* and *Rhizoctonia solani* in dual culture tests. The biological properties of genus *Pseudomonas* are considered superior because of their adaptive metabolism and their ability to produce a diverse array of potent antifungal metabolites. These include simple metabolites such as 2, 4-diacetylphloroglucinol, phenazine-1-carboxylic acid and pyrrolnitrin [3-chloro-4-(2'-nitro-3'-chlorophenyl)-pyrrole], as well as the complex macrocyclic lactone, 2, 3-de-epoxy-2, 3-didehydra-rhizoxin. Pyrrolnitrin is active against *Rhizoctonia* spp, *Fusarium* spp, and other plant pathogenic fungi, and it has been used as a lead structure in the development of a new phenylpyrrole agricultural fungicide (Ligon *et al*. 2000) [58]. Strains of *Pseudomonas fluorescence* also have the potential to produce known secondary metabolites such as siderophore, HCN and protease that showed antagonistic activity against *Macrophomina phaseolina, Rhizoctonia solani, Phytophthora nicotianae, Pythium* sp. and *Fusarium* sp. (Ahmadzadeh *et al*. 2006 [59]; Singh *et al*. 2014 [51]).

ACC-Deaminase Activity

Ethylene is essential for the growth and development of plants, but it has different effects on plant growth depending on its concentration in root tissues. At high concentrations it can be harmful, as it induces defoliation and cellular processes that lead to inhibition of stem and root growth as well as premature senescence, and also causes to decreased vegetative period, all of which lead to reduced crop performance (Lie *et al*. 2005)[60]. Under different types of environmental stress, such as cold, drought, flooding, infections with pathogens and presence of heavy metals; plants respond by synthesizing 1-aminocyclopropane-1-carboxylate (ACC), which is precursor for ethylene (Glick *et al*. 2007)[61]. The increase of ethylene in plants is directly related with the concentration of ACC in plant tissues (Machackova *et al*. 1997)[62]. The reduced levels of ACC result in low synthesis of endogenous ethylene, which lessens the inhibitory effects of higher ethylene levels (Yuhashi *et al*. 2000)[63]. Recently, it has been reported that certain PGPR also have ACC-deaminase activity that changes ACC into alpha-keto-butyrate and ammonia (Arshad *et al*. 2007)[64] and thereby lower the level of ethylene in the plant (Penrose *et al*. 2001)[65]. Rhizobacteria with ACC-deaminase activity are found to belong to genera *Achromobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas* and *Rhizobium* (Govindasamy *et al*. 2008 [66]; Duan *et al*. 2009 [67]). More specifically, the soil borne fluorescent pseudomonads have gained particular attention throughout the global scene because of their catabolic versatility, excellent root colonizing ability and their capacity to produce a wide variety of enzymes and metabolites that favour the plant to withstand varied biotic and abiotic stress conditions (Patten and Glick 2002 [15]; Vivekananthan *et al*. 2004 [68]; Mayak *et al*. 2004 [69]). In 1998, Glick and coworkers suggested a model explaining how ACC-deaminase containing PGPR can lower plant ethylene levels and in turn stimulate plant growth. According to this model, PGPR attach either to seed surface or roots of developing plant, in response to tryptophan and other amino acids produced by the seeds, and thus synthesize the auxin (IAA) (Patten and Glick 2002) [15]. Together with the plant produced IAA, the bacterial IAA stimulates synthesis of ACC-synthase, which is responsible for the rapid transformation of S-adenosyl-L-methionine into ACC (Li *et al*. 2000) [70]. Besides, plants inoculated with PGPR having ACC-deaminase are more

Table 2. Examples of PGPR tested for various crop types

PGPR	Plant	Conditions	Results	References
Pseudomonas putida, *Azospirilium*, *Azotobacter*	*Cynara scolymus*	*In vitro*	Phosphate solublizing bacteria along with nitrogen fixing bacteria led to significant increase in radicle and shoot length, shoot weight, coefficient of velocity of germination, seedling vigority index, and significant decrease in mean time of germination	Jahanian *et al*. 2012[111]
Pseudomonas sp. PS1	*Vigna radiata* (L.) wilczek	Pots	Significantly increased plant dry weight, nodule nubers, total chlorophyll content, leghaemoglobin, root N, shoot N, root P, shoot P, seed yield and seed protein	Ahemad and Khan 2012e[105]
Bradyrhizobium MRM6	*Vigna radiata* (L.) wilczek	Pots	When herbicide tolerant *Rhizobium* strain MRP1 was used with herbicide, it increased the growth parameters at all tested concentrations of herbicides (quizalafop-p-ethyl and clodinafop)	Ahemad and Khan 2012f [48]
Pseudomonas putida (PP) and *P. fluorescens* (PF)	*Hyoscyamus niger*	Pots	Inoculation of *H.niger* plants by PP-168 and PF-187 remarkably improved the seeds alkaloids content and yield.	Ghorbanpour *et al*. 2013[112]
Pseudomonas fluorescens and *Bacillus subtilis*	*Vigna mungo*	Pots	Maximum number of leaf, plant height, shoot length, root length and roots were observed in the plants treated with *Pseudomonas fluorescens* + *Bacillus subtilis* than those of other.	Kannahi and Kowsalya 2013[49]
Bacillus amyloliquefaciens, *Serratia marcescens* and *B. pumilus*	*Camellia sinensis* (L.) O. Kuntze	Nursery and field	Application of PGPR led to the enhancement in activities of defense related enzymes such as phenyl alanine ammonia lyase, peroxidase, chitinase and ß-1,3 glucanase.	Chakraborty *et al*. 2013[111]
Bacillus pumilus and *Pseudomonas pseudoalcaligenes*	*Oryza sativa* L.	Field	Plants inoculated with PGPR under saline conditions showed higher germination, survival, dry weight and plant height; increased concentrations of N, P, K and reduced concentrations of Na and Ca as compared to non-inoculated control plants under saline conditions.	Jha and Subramanian 2013[113]
Pseudomonas sp.	*Cicer arietinum* L.)	Plate test	Chickpea seeds bacterized with *Pseudomonas* sp. PGPR 2 showed a significant increase in percentage seed germination followed by PGPR 3 and LK 884 in *desi* variety PBG1 whereas, seed germination percentage was maximum in *kabuli* variety, BG 1053 when inoculated with reference culture LK884 followed by PGPR 3 and PGPR 2.	Kaur and Sharma 2013[109]
Pseudomonas fluorescens Alcaligenes, *Staphylococcus*, *Agrobacterium*, *Pantoea* and *Bacillus*	*Aphis gossypii* Strawberry (*Fragaria x ananassa Duch.) cv.Aromas*	Pots Greenhouse	Treated plants had a significant increase in yield weight of (approx. 58%). All of bacterial treatments significantly increased fruit yield, number and weight. Except for magnesium and zinc in the leaf, the concentrations of all plant tissue nutrients significantly increased by bacterial treatments tested.	Fahimi *et al*. 2014[103] Ipek *et al*., 2014 [114]

resistant to the injurious effects of the stress ethylene that is produced as a result of stressed environments (Nadeem *et al*. 2007) [71].

Synergistic effects of PGPRS on the symbiotic efficiency of *Rhizobium*

Nitrogen fixation and nodulation

Molecular N or dinitrogen (N_2) makes upto one fifth of the atmosphere, but is metabolically unavailable directly to higher plants or animals (Saikia and Jain 2007)[72]. Biological nitrogen Fixation (BNF) can contribute to the replenishment of soil N, and reduce the need for industrial nitrogenous fertilizers (Lanier *et al*. 2005)[73]. Thus, it is made available to plants by the microorganisms through the process of symbiosis. The interaction of rhizobia with roots of leguminous plants results in establishment of effective N_2 fixing symbiosis (Table 2). In this process, rhizobia reduce atmospheric N to ammonia using enzyme nitrogenase and supply this essential nutrient to the host plant cells. It is an energetically unfavourable reaction, carried out by prokaryotic microorganisms including bacteria, cyanobacteria and actinomycetes, in symbiotic or non-symbiotic association with plants (Giller 2001)[74]. Nodules are the sites of symbiotic nitrogen fixation. Although most *Rhizobium* isolates can nodulate more than one host plant species, while several different bacterial species are often isolated from a single legume (Young and Haukka 1996)[75]. The exchange of chemical signals between compatible strains of *Rhizobium* and legumes has been named as molecular dialogue (Cooper 2007)[76], which serves as an initiate of the nodule development (Murray *et al*. 2007)[77]. In the legume rhizosphere, the rhizobia become affected by the chemotactic and growth promoting compounds. The combined effect results in root colonization. Establishing a fully functional symbiosis needs a successful

Table 3. Compatibility of PGPRS with *Rhizobium in vitro*

PGPR	*Rhizobium* sp.	Crop	Results	References
Azospirillum lipoferum	*R. leguminosarum* bv. *trifolii*	*Trifolium repens*	Improved nodulation.	Tchebotar *et al*. 1998[115]
Azospirillum lipoferum	*R. leguminosarum* bv. *trifolii*	*Cicer arietinum*, *Cajanus cajan*	Improved nodulation.	Deanand *et al*. 2002 [116]
Azospirillum	*Rhizobium*	*Phaseolus vulgaris L.*	Increase fixed quantity.	Remans *et al*. 2008[117]
Combined inoculation of *Azotobacter*, *Azospirillum*, *Pseudomonas*	*Mesorhizobium*	*Cicer arietinum* L.	Promotion of grain yield and biomass.	Rokhzadi *et al*. 2008[118]
Azospirillum brasilense	*Rhizobium*	*Glycine max* L., *Phaseolus vulgaris*, *Zea mays* L.,	Increased phytohormones, vitamins and siderophore production.	Dardanelli *et al*. 2008[119]; Cassan *et al*. 2009[120]
Azotobacter chroococcum	*Mesorhizobium ciceri*	*Cicer arietinum* L.	Improved growth, nodulation and yield.	Qureshi *et al*. 2009[121]
Pseudomonas fluorescens WSM3457	*Ensifer* (*Sinorhizobium*) *medicae* WSM419	*Medicago truncatula* cv. *Caliph*	Enhanced nodulation and symbiotic effectiveness of *Medicago truncatula*.	Fox *et al*. 2011[122]
Pseudomonas aeruginosa	*Mesorhizobium* sp.	*Cicer arietinum* L.	Nodulation, root and shoot dry weight, grain and straw yield, nitrogen and phosphorus uptake were significantly increased.	Verma *et al*. 2013[123]
Azotobacter chroococcum, *P. aeruginosa* and *Trichoderma harzianum*	*Mesorhizobium*	*Cicer arietinum* L	Plant growth along with the antagonistic activities against *F. oxysporum* and *R. solani*.	Verma *et al*. 2014[124]

completion of many steps that start from recognition signals exchanged between the plant and bacteria and end in the differentiation and operation of root nodules. Gage (2002)[78] proposed a model for the growth of *S. meliloti* in infection thread produced in alfalfa root hair cells. He used mixed populations of *S. meliloti* L5-30 and marked them with constitutively expressed green fluorescent protein or DsRed. A good number of infection threads were found to be infected by cells of both populations, which produced green and red regions in the same infection thread. It is well accepted all over the scientific world that nodule formation requires oxidative stress and rhizobial response to it. This is how a plant controls the abortion of infection thread development and consequently, controls the nodule number (Prell and Poole 2006)[79]. Exposure to light was also found to be involved in suppressed nodulation in *P. sativum* L. cv. *sparkle* roots (Lee and Larue 1992)[80]. Nodulation is also affected by the application of mineral fertilizers. Rahman and co-workers (2002)[81] stated that increased NPK dose resulted in reduced nodulation in *Samanea saman* seedlings, while K had little effect. Production of siderophores in the soil also plays a significant role in enhancing nodulation and consequently biological nitrogen fixation because the nitrogenase enzyme requires a lot of iron (Catellan *et al*. 1999)[82]. In addition to other factors, plant hormones have very important regulatory role in the establishment and development of nodulation (Frankenberger and Arshad 1995)[83]. Plant growth regulators synthesized by microorganisms could have important role in symbiosis, especially in nodulation during legume-*Rhizobium* interaction (Hirsch *et al*. 1997)[84], or in direct plant growth promotion (Kobayashi *et al*. 1993)[85]. Ethylene is also found to be involved in affecting rhizobial nodulation in legumes (Glick 2005)[86]. A successful symbiosis and nitrogen fixation may be achieved, if the conditions of rhizobial inoculants remain optimized (Zahran 2001)[87]. Moreover, total plant weight and total N of lentil

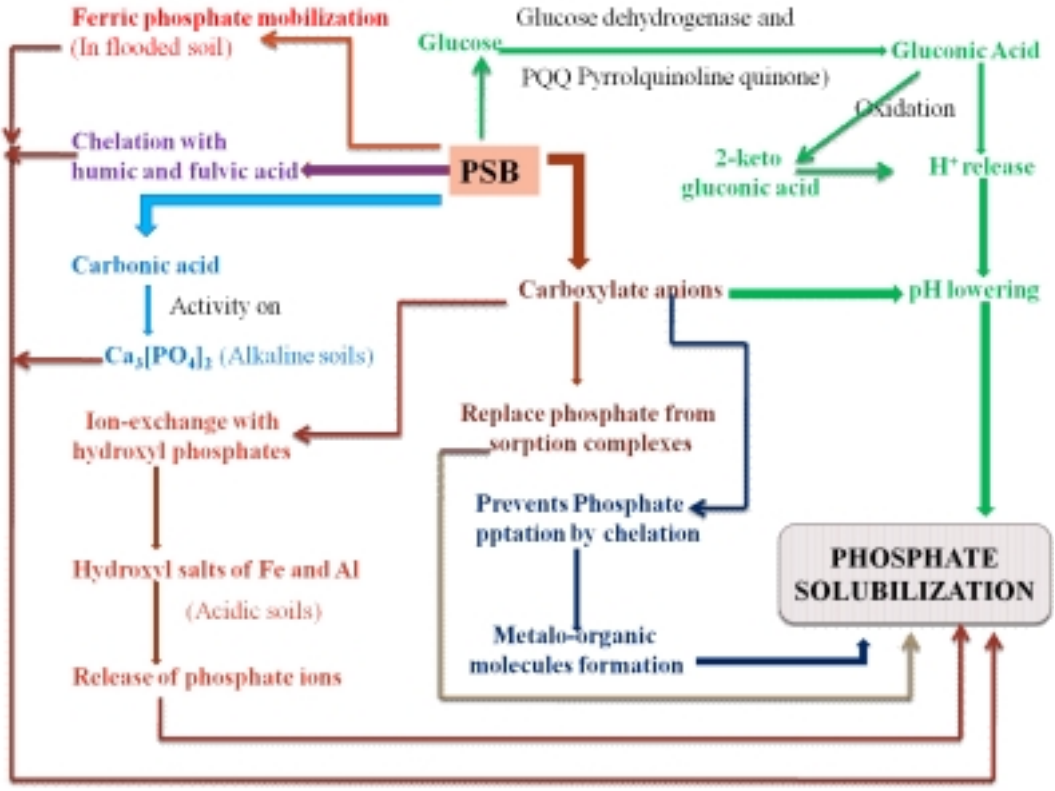

Fig. 1. Various organic/inorganic substances produced by PSB responsible for phosphate solubilization in soils

have been observed to be highly correlated (Shah *et al.* 1994)[88]. Miller *et al.* (2007) [89] carried out a study regarding application of *Rhizobium* as inoculum. They stated that the strains of *Rhizobium leguminosarum* bv. *trifolii* produced effective nodules on *Trifolium ambiguum* and ineffective nodules on *Trifolium repensi*. Beneficial plant-microbe interactions in the rhizosphere contribute a lot towards plant health and soil fertility (Jeffries *et al*. 2003)[90]. Efficiency of external inputs could be increased by the selection of the best combinations of beneficial microbes for sustainable agricultural production. Saini and Khanna (2012)[91] observed that co-inoculation with *Pseudomonas* spp. and *Rhizobium* had significantly improved root length, total biomass, yield and nodulation in lentil.

Compatibility of PGPR with *Rhizobium in vitro*

Application of PGPR for improvement of crops has also been investigated for many years, with recent attention focused on co-inoculation with rhizobia and PGPR with different growth attributes for growth promotion (Table 3). Populations of bacteria have functional roles within communities that permit their survival. Distinct microbial populations in rhizosphere frequently interact with each other. Syntrophic relationships between different organisms have been demonstrated in several microbial ecosystems. Bacteria live in consortia bound to surfaces such as in biofilms, flocs or granules. Under these conditions the bacteria are positioned in a heterogeneous environment. It is increasingly apparent that in nature, bacteria function less as individuals and more as coherent groups that are able to inhabit multiple ecological niches. When these strains are made into an inoculum consortium, each of the constituent strains of the consortium not only out-competes with the others for rhizospheric establishments, but complement functionally for plant growth promotion. Prasad and Chandra (2003)[92] demonstrated the increase in viable population of *Rhizobium* when co-cultured with PSB or/and PGPR. Pandey and Maheswari (2007)[93] described the relationship between two distantly related isolates, *Burkholderia* sp. MSSP and *Sinorhizobium meliloti* PP3. They discovered that in combination both the strains promote growth of host plants because of increased indole-3-acetic acid (IAA) production and phosphate solubilization than single inoculation under laboratory conditions. About 25% increase in mean growth rate was recorded for *S. meliloti* PP3 when grown in mixed-species, two-species culture with respect to single species culture. This interaction also indicates that in soil, association with *Burkholderia* sp. MSSP favours *S. meliloti* PP3 as an adaptation of high rate of reproduction-a well-known strategy that enables organisms to successfully survive and maintain themselves in communities. Seneviratne (2003)[94] has mentioned that co-inoculation and co-culture of microbes have been observed to perform the tasks better than the individual microbes.

Introduced organisms are highly stressed, alien to the natural soil environment, and often physiologically not ready to compete in soil with the indigenous species that have adapted to the ecological niche over several generations. Many inoculant formulations specifically address these issues by incorporating microorganisms into carriers enriched with selective food sources, suppressants for indigenous species, buffers and other ingredients, which can transiently alter the microphysical environment of the soil to provide a temporary safe haven for the introduced species. A successful formulation must have increased shelf life, should not be phytotoxic to the crop plants, should dissolve well in water and release the bacteria, tolerate adverse environmental conditions, be cost effective and give reliable control of plant diseases, be compatible with other agrochemicals, carriers must be cheap and readily available for formulation development (Jeyarajan and Nakkeeran 2000)[95]. Talc and charcoal based formulations are common in practice (Nakkeeran *et al*. 2004 [96]; Vivekananthan *et al*. 2004 [68]; Sharma 2008 [97]; Gangwar *et al*. 2014 [98]). The population load of *P. putida* strains at the end of 6th month was 10^8 cfu/g of the product (Bora *et al*. 2004) [99]. Shelf life of various PGPR species in peat (Kavitha *et al*. 2003 [100]; Nakkeeran *et al*. 2004 [96]) and vermiculite-based formulation (Saleh *et al*. 2001) [101] retained for more than six months and 10 months. Sahai (2008) [102] reported that talc powder and vermiculite were better carriers than

aluminum silicate, in pot trial experiments on wheat and *Vigna mungo*.

CONCLUSION

With the increasing problems associated with the use of synthetic chemicals in agriculture (impacts on health and the environment, resistance development in plant pathogens and pests, etc.), there has been an ever-increasing interest in the use of native and non-native beneficial microorganisms to improve plant health and productivity while ensuring safety for human consumption and protection of the environment. These microorganisms are the potential tools for sustainable agriculture and the trend for the future. Along the same line, biotechnology can be applied to further improve strains that have prized qualities by creating transgenic strains that combine multiple mechanisms of action. The use of mixed biofertilizers is advocated to get the maximum benefits due to additive and synergistic effect. Overall, beneficial microorganisms, such as those reviewed in this work, have demonstrated multifaceted beneficial effects pertaining to increased plant growth and health. However, more studies on the precise mode of action and the ecophysiology of these microorganisms in relation to other soil borne inhabitants may well help in the timely and appropriate use of these organisms. Efforts should be directed towards motivating products that are based on local isolates, as biofertilizer effectiveness is dependent upon factors like plant type, soil type, soil pH and climatic conditions. Further, detailed studies are needed on the community composition and conditions *viz*. population, effect of cultivar on bacterial population dynamics, influence of inoculum density on antagonistic activity, survival of inoculum under adverse conditions and role of environmental conditions in altering the activity of rhizobacteria. The detailed knowledge on molecular signaling mechanisms between related strains and species also needs to be understood for the development of a better formulation that could suppress a broad spectrum of pathogens and pests besides plant growth promotion. Moreover, the confusion related to use of certain potential biocontrol agents such as *Pseudomonas aeruginosa*, *P. cepacia* and *Bacillus cereus* that behave as opportunistic human pathogens, also needs to be solved.

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