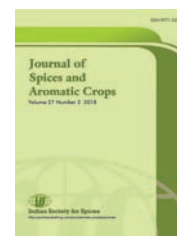


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## Understanding plant-beneficial microbe interactions for sustainable agriculture

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### Abstract

A better understanding of the specific molecular interactions between plants and microbes is crucial to develop newer strategies for sustainable agriculture. The productivity of wide range of agricultural crops under decreasing land resources and shrinking biological potential of the soil need to be improved. Search for useful microorganisms associated with the plants has been highly productive for sustainable agriculture. We take a close look at the current level of molecular interactions that mostly involve specific molecular patterns of microbes and their cognate receptors in plants and development of efficient biofertilizers for improving crop yields. This article covers the broader aspects of plant-microbe interactions with more focus on plant growth promoting rhizobacteria (PGPR). Further upcoming strategies to understand the plant-PGPR interactions are discussed.

**Keywords:** biofertilizers, growth promotion, induced resistance, plant-microbe interaction, sustainable agriculture

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### Introduction

Plants interact with a variety of microorganisms that influence the annual crop production, and at times threaten global food security. We also have several beneficial effects of such interactions that are widely known like symbiotic nitrogen fixers and arbuscular mycorrhiza. The question here is how do plants differentiate beneficial and harmful microbes. To reduce the severity of crop losses due to the damaging effects of pathogenic microbes, antifungal and antibacterial substances are routinely used in agriculture. The prohibitive costs and the rapidity with which the microbial

pathogens develop resistance increased consumer awareness on the detrimental effects of synthetic chemicals and their continued use (Neeraja *et al.* 2010).

The plants are able to sense evolutionarily conserved molecular pattern from microbes called microbe-associated molecular patterns (MAMPs, referred as PAMP when it originates from a pathogenic microbe) and activate responses inside the cell. These molecules are recognized by either receptor-like proteins or receptor kinases called pattern recognition receptors (PRRs) mostly localized to the plasma-membrane.

The never-ending molecular arms race of plants against microbial pathogens, involving a multitude of PAMPs, PRRs, effectors, R proteins, *via* the immunity pathways are referred to as pathogen-triggered immunity (PTI) and effector triggered immunity (ETI). These pathways may hold a key to develop alternative strategies to induce disease resistance in plants. A promising and versatile PAMP, for which detailed structure/function relationships and the molecular mode of action are currently being revealed, are chitin and chitosan oligo- and polymers (Moerschbacher 2005; Yin *et al.* 2010).

Chitin is the second most abundant natural polysaccharide consisting of  $\beta$  (1, 4)-linked N-acetyl-D-glucosamine (GlcNAc) units in a linear form. Chitin is a major constituent of fungal cell walls as well as of insect exoskeletons and as such, chitin is a prominent PAMP indicating the presence of potentially detrimental organisms to plants. Chitin is insoluble in water and mainly exists in two crystalline ( $\alpha$ - and  $\beta$ -) forms (Purushotham & Podile 2012). Chitinases (EC 3.2.1.14) hydrolyze chitin to NAG, chitobiose, or smaller chitooligosaccharides (CHOS). Chitinases are found in a wide range of organisms, including bacteria, fungi, plants, animals and are closely associated with the physiological roles of their substrates. The CHOS are gaining interest in the food, agriculture, and medicine-related industries in light of the diverse applications of these molecules. Most biological activities require CHOS with degree of polymerization (DP) $>4$ , but the synthesis of oligomers with  $>6$  DP has been a daunting task. The use of CHOS as a broad-spectrum vaccine against plant diseases highlights the need to produce specific CHOS for crop protection and production (Yin *et al.* 2010).

### Different types of plant-microbe interactions

The interactions between plant and microbes can be either beneficial, neutral or harmful. The outcome is dependent on the delicate balance among soil and plant type. Beneficial microbes include nitrogen fixers (NF), phosphate solubilizing bacteria (PSB), vesicular-arbuscular mycorrhiza (VAM), and plant growth promoting rhizobacteria (PGPR). Mostly, these microbes are in a mutual relationship with plants for carbon source and simultaneously improve plant

growth by various mechanisms. Few microbes in soil have no relation to the plant processes. Neither they get to benefit from the plant nor they provide benefit to the plant; such organisms could be classified under the neutral category of microbes. The harmful microbes referred as phytopathogens invade plants for their own benefit to get nutrients or to propagate, resulting in decrease in crop yield. Phytopathogens cause severe damage to plant developmental processes that sometimes lead to the death of plants. The estimated global crop loss was about 12.5% due to pathogenic microbes attack on plants (Oerke, 2006). Examples of different pathogenic microbes are *Ralstonia solanacearum* (bacteria) causing wilt in tomato, *Aspergillus niger* (fungi) causing crown rot in groundnut, soybean mosaic virus (virus) causing necrosis in soybean *etc.*

### Nitrogen fixers

Nitrogen-fixing bacteria are either in symbiotic or non-symbiotic association with the host plants. Rhizobia are well-known symbiotic NF of legumes and they fix approximately 50-300 kg N/ha (Mahdi *et al.* 2010). Rhizobia successfully form root nodules involving a classical example of exchange of chemical signals between plants and bacteria. Flavonoids like genistein, naringenin, and hesperetin from plant root exudates (REs) are reported as the signalling molecules to activate the *nod* genes in rhizobia, responsible for production of nod factors. Further, the nod factors take the form of lipo chito oligosaccharides (LCOs) and act as the prime molecules for plant-rhizobia specificity. A  $\text{Ca}^{+2}$  dependent receptor kinase system of plasma membrane gets activated in the development of the nodule to maintain a symbiotic relationship (Coskun *et al.* 2017). Free-living *Azotobacter* and *Azospirillum* also fix nitrogen by an associative symbiotic relationship in the soil. They can fix up to 20-40 kg N ha<sup>-1</sup> (Mahdi *et al.* 2010) and a specific host plant is not required for their association. Maize, sorghum, sugarcane, wheat, and pearl millet are mostly recommended by this type of NF (Rajae *et al.* 2007; Gholami *et al.* 2009). The NF increase soil fertility, seed germination, plant growth, and produce antibiotics against pathogens. Another group of NF is blue-green algae/cyanobacteria which can fix up to 20-30 kg N ha<sup>-1</sup> in rice fields. They are typically found in the rice fields, hence named as paddy organisms

(Nayak *et al.* 2004). *Azolla*, a free-floating nitrogen-fixing fern is in symbiotic association with *Anabaena*, alleviates P, S, K, Zn, Mb, and Fe (nutrients) deficiency to the host plant (Yatazawa *et al.* 1980). This *Azolla*-*Anabaena* complex is also used as a nitrogen fertilizer along with cyanobacteria to increase rice yield (Watanabe *et al.* 1977).

#### *Phosphate solubilizing bacteria*

Mostly phosphate in soils is available in bound form (di/tricalcium phosphate, hydroxyapatite, and rock phosphate) which cannot be absorbed by the plants. PSB can solubilize the bound phosphate and make it available to plants without using phosphate fertilizers. As a first step, PSB secrete organic acids by utilizing sugars from the plant REs and decrease the soil pH (Goswami *et al.* 2016). Later, the acid phosphatases, released by PSB, mineralize the bound phosphate for plants' uptake (Mahdi *et al.* 2010; Sharma *et al.* 2011). *Aerobacter*, *Achromobacter*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, and *Rhizobium* are the few PSB. For example, *Pseudomonas fluorescens* and *Bacillus megaterium* with phosphate-solubilizing ability increased the plant growth and P, N levels in *Cicer arietinum* and *Phaseolus vulgaris* (Sharma 2007; Collavino *et al.* 2010). PSB like *Pantoea* sp., *Enterobacter* sp., *Burkholderia* sp., and *Sphingomonas* sp. can also phytostabilize heavy metals (like lead and aluminum) along with phosphate solubilization in rice seedlings (Park *et al.* 2011; Panhwar *et al.* 2014). This indicates the advantage of using PSB in different situations like plant growth and phytostabilization.

#### *Vesicular-arbuscular mycorrhiza*

The Glomeromycotaphylum fungi penetrate into the vascular plant roots involving chemotaxis mediated by molecules like strigolactones and cutin of plant REs (Venturi & Keel 2016). Such chemoattractants in the REs induce AMF to produce Myc-LCOs factors which develop vesicles and arbuscules in plant roots through a process similar to the plant-rhizobial symbiosis. The AMF capture nutrients (P, N, and S) from the soil and provide to plants for their growth. This symbiotic relationship between fungi and plant root system was earlier referred as

mycorrhizae. The arbuscular mycorrhizal fungi (AMF) form a sheath like protective cover around roots to protect plants from different environmental conditions like drought, salinity, other pathogenic fungal attacks. They increase the root elongation rate and improve the capturing ability of less available or inaccessible mineral nutrients (Zn, Co, Ca, and Mo) from the soil. *Artemisia annua* inoculated with AMF significantly increased plant growth, essential oil, and artemisinin content (Kumar *et al.* 2017).

A consortium of beneficial microbes was applied to test its effect on crop yield (Zaidi *et al.* 2017). The consortium may exhibit positive or negative effects on plants, depending on the nature of interaction between the members of the consortium. Mostly combined use of beneficial microbes resulted in increased plant growth, yield and enhanced biocontrol potential towards pathogens than their individual application (Jain *et al.* 2015a; Jain *et al.* 2015b; Sarma *et al.* 2015). Alagawadi & Gaur (1994) first used the dual inoculum of *Azospirillum brasilense* (NF) and *Pseudomonas striata* or *Bacillus polymyxa* (PSB) on sorghum. This application increased grain yield by improving N and P absorption by the plants. In *Rhizobium* and PSB dual application, yield increased by 20% in comparison to their individual use in wheat (Afzal & Bano 2008). Dual inoculation of PSB and *Glomus fasciculatum* also increased alfalfa plants growth (Piccini & Azcon 1987). *Rhizobium leguminosarum* and AMF dual inoculation on faba bean, provided tolerance to alkaline conditions along with improved plant growth (Abd-Alla *et al.* 2004). This consortium of beneficial microbes is presently used in the restoration of the degraded landscape. For example, the mass multiplication of beneficial AMF, NF, and rhizobacteria in 30 legume species restored the soil fertility (Ghosh & Dutta 2016). Out of all the tested legumes, *Arachis hypogaea*, showed the highest colonization by this tri-partite symbiotic relationship along with increased yield in elevated drought stress. These NF or PSB or AMF associations provide benefit to plants by increasing the nutrient availability and alleviate some environmental stress conditions.

#### *Plant growth promoting rhizobacteria (PGPR)*

Bacteria that provide benefit to plant can be

symbiotic or free-living in the soil. They are abundant near the roots. The fraction of soil influenced by roots is called as rhizosphere (Parray *et al.* 2016). The term rhizosphere was first coined by a German plant physiologist and agronomist Lorenz Hiltner in 1904. In Greek the word 'rhizo' means root. The rhizosphere is divided into three zones: ectorrhizosphere- soil near the root, rhizoplane- surface of the root and endorhizosphere- inside the root tissue, including cortical layers and endodermis (Badri & Vivanco 2009; Oburger & Schmidt 2016). The rhizosphere is colonized by a diverse group of microorganisms than the surrounding bulk soil. Approximately  $10^{10}$ - $10^{12}$  microflora will be present in a gram of rhizosphere soil, which is 1000-2000 times higher than the bulk soil microbial population. Some of the rhizobacteria in the soil promote plant growth, yield, and control diseases. Such free-living beneficial bacteria are termed as plant growth promoting rhizobacteria (PGPR) (Podile & Kishore 2006). About 2-5% of the total rhizosphere bacteria constitutes PGPR (Antoun 2006). Strains of the genera characterized as PGPR are *Acetobacter*, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Clostridium*, *Derxia*, *Enterobacter*, *Exiguobacterium*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Methylobacterium*, *Ochrobactrum*, *Pantoea*, *Paenibacillus*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Stenotrophomonas*, and *Zoogloea* (Podile & Kishore 2006; Chauhan *et al.* 2015; Jha & Saraf 2015).

### Classification of plant growth promoting rhizobacteria

PGPR are classified on the basis of functional and physiological aspects. Based on function, Somers *et al.* (2004) classified PGPR as biofertilizers, phytostimulators, rhizoremediators, and biopesticides. This was less accepted as the functions of PGPR are overlapping with each other. Whereas, Gray & Smith (2005) classified PGPR into two simple groups based on their colonization ability. The intracellular PGPR (iPGPR): for bacteria colonizing inside the root (also known as endophytes) and extracellular PGPR (ePGPR): for bacteria colonizing in the rhizosphere, rhizoplane or intercellular spaces of the root. Rhizobial interaction with legumes is a simple example of iPGPR. They form nodules

in the root and fix the atmospheric N and promote plant growth. The gram-negative, rod-shaped bacterial population is dominant in iPGPR than gram-positive, cocci, rods or pleomorphic bacteria. The ePGPR are not able to form nodules, but they colonize roots and influence plant nutrient uptake, growth, and yield by an array of direct and indirect mechanisms (Fig. 1). Some PGPR directly regulate the plant processes by impersonating synthesis of plant hormones, increasing soil minerals availability, as a way to enhance growth directly (Persello-Cartieaux *et al.* 2003; Taurian *et al.* 2010). Whereas, other groups of PGPR contribute indirectly by providing biocontrol against pathogens. They compete with pathogenic microorganisms for nutrients and niche (Dutta & Podile 2010), produce lytic enzymes and antimicrobials (Kavino *et al.* 2010; George *et al.* 2015) and induce systemic resistance (Tjamos *et al.* 2005) to kill the pathogens.

### Direct plant growth promoting mechanisms

#### *Facilitating nutrient acquisition*

PGPR facilitate the uptake of mineral nutrients like nitrogen, phosphate, iron, and zinc from the soil by converting the nutrients to soluble form. Nitrogen is a vital mineral for many living organisms including plants. It is required for the synthesis of building blocks like nucleotides, DNA, RNA, amino acids, and proteins *etc.* In the atmosphere, nitrogen is available in diatomic form ( $\text{N}_2$ ) with a strong triple bond. This form of nitrogen is inert and won't be able to react with any other chemicals as well as non-absorbable by plants or animals. Nitrogen-fixing PGPR convert atmospheric nitrogen into ammonia and contribute to the N requirement of the plants. All nitrogen-fixing PGPR possess metalloenzymes known as nitrogenase, coded by *nif* genes. They include structural genes, iron protein activating genes, iron-molybdenum cofactor biosynthesis genes and regulatory genes necessary for the nitrogen fixation. Nitrogen-fixing *Pseudomonas* strain K1 increased grain yield and shoot biomass of two basmati rice varieties in comparison to non-nitrogen fixing *Zoogloea*, *Azospirillum brasilense*, and *Azospirillum lipoferum* (Mirza *et al.* 2006). A similar result was observed by Kuan *et al.* (2016) in maize with nitrogen-fixing *Klebsiella* sp., *Bacillus pumilus*, and *Acinetobacter* sp. inoculation.

After nitrogen, phosphorus is the second most essential macronutrient required for the growth of plants. Mostly phosphate exists in insoluble form, even in phosphate-rich soils. PGPR or PSB secrete organic acids (gluconic, glycolic, malonic, oxalic, and succinic acid) and phosphatases to disturb soil phosphate dynamics. They convert insoluble phosphates into soluble mono or di basic ( $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$ ) ions, referred as mineral phosphate solubilization (Mallick 2016). This leads to an increase in phosphate availability in rhizosphere and plants phosphate utilization. *Bacillus*, *Enterobacter*, *Erwinia* and *Pseudomonas* spp. are the most potent PSBs (Gyaneshwar *et al.* 2002). As an example, a gluconic acid producing endophytic *Pseudomonas* sp. improved growth and yield of *Pisum sativum* L. in phosphate-limiting conditions (Oteino *et al.* 2015).

Like phosphate, zinc also exists as insoluble complexes in soil and cannot be utilized by the plants. PGPR fulfil the plant's zinc requirement by solubilizing zinc complexes and releasing zinc into the rhizosphere. Zinc solubilization is achieved by production of organic acids, inorganic acids (sulphuric acid, nitric acid, and carbonic acid), chelating ligands, proton extrusion, and/or with the oxidoreductive systems present on the cell membranes of PGPR (Wakatsuki 1995; Saravanan *et al.* 2004; Goteti *et al.* 2013).

#### Phytohormones production

Phytohormones are plant growth regulators that influence plant development; produced by plants, algae, and few prokaryotic microorganisms. There are five important phytohormones, i.e. auxins, gibberellic acids, cytokinins, abscisic acid, and ethylene. PGPR secreted phytohormones influence the root zone and the plant developmental process. For instance, indole-3-acetic acid (IAA) is an auxin that controls many important plant physiological processes, that include cell elongation, cell division, tissue differentiation, lateral root formation, and response to light and gravity (Parray *et al.* 2016). PGPR with IAA producing capability will have a capability to regulate these developmental processes by adding IAA to the plant's auxin pool (Vessey 2003). Plant's potential to absorb nutrients and water depends on the root surface

area. The increase in root surface by IAA improves absorption capacity from large volume of soil and their utilization for plant growth (Volkmar & Bremer 1998).

Gibberellic acids (GA) are tetracyclic diterpene compounds that promote seed germination, sex expression, stem elongation, flowering, and senescence in plants. They are produced by higher plants, bacteria, and fungi. All the GAs available in the rhizosphere are not biologically active. PGPR with potential to deconjugate gibberellin-glucosyl bonds can generate active dihydroxylated GAs (like GA1, GA3, and GA4) in the root zone. This active form of GAs promotes plant growth. For example, *Bacillus cereus*, *B. macrolides*, and *B. pumilus* significantly increased red pepper growth by producing biologically active GAs (Joo *et al.* 2004). A new PGPR, *Leifsoniasoli* SE134 also influenced the growth and yield of cucumber, rice, tomato, and radish by producing active GAs (Kang *et al.* 2014). The third group of phytohormones, cytokinins are involved in cell division of root and shoot formation. *Arabidopsis thaliana* mutants lacking cytokinin signalling genes (*Cre1*, *Ahk2* and *Ahk3*, and *Rpn12*) showed impaired plant growth in presence of plant growth promoting *Bacillus megaterium*. This shows the complementary role of cytokinin and PGPR in plant growth promotion (Ortiz-castro *et al.* 2008). Other two phytohormones abscisic acid (ABA) and ethylene are stress-tolerant hormones. ABA influences seed dormancy and bud growth, whereas ethylene affects the cell shape and growth. *A. thaliana* elevated salt stress in presence of *Azospirillum brasilense* and showed a two-fold increase of plant ABA content (Cohen *et al.* 2008). This explains the importance of ABA in stress tolerance along with growth promotion. Plant internal ethylene levels are triggered by many biotic and abiotic factors. The increased ethylene levels inhibit plant growth by hindering DNA synthesis and cell division processes. PGPR are able to synthesize 1-aminocyclopropane-1-carboxylate (ACC) deaminase can counteract the negative effects of ethylene *via* decreasing its levels. The ACC deaminase cleaves ACC, the precursor of ethylene into ammonia and  $\alpha$ -ketobutyrate. *Pseudomonas putida* inoculated *Papaver somniferum* plants resisted against the negative effects of downy mildew (caused by *Peronospora* sp.) by

decreasing ACC levels, increasing IAA and significantly improved the plant growth. Rkh1-Rkh4 PGPR, isolated from weed rhizosphere showed significant growth promotion of soybean by secreting IAA, GA, and ABA to elevated salt stress (Naz *et al.* 2009), indicating the interlink of phytohormones secreted by PGPR in regulating growth and stress alleviation in plants.

#### *Siderophores production*

Iron is an important micronutrient and serves as a cofactor for many redox maintaining enzymes of cell. Iron is available in insoluble ferric hydroxide form in soils. This limits the iron availability even in iron-rich regions for proper plant growth. Siderophores are low molecular weight, small, iron chelating compounds released by the bacteria (including PGPR), fungi and plants. The siderophores have a high affinity to  $Fe^{3+}$  and form  $Fe^{3+}$ -siderophore complexes that are absorbed by the plants. Plants have adapted mechanisms to absorb  $Fe^{3+}$  bound to the bacterial siderophores (Masalha *et al.* 2000) by ligand exchange process. PGPR with siderophore producing ability can chelate iron in the soil and make it available to plant, and limit its availability to pathogens that cannot produce affinity siderophores. This generates a competition between PGPR and pathogen for iron utilization. Further, they suppress pathogens by inducing defense mechanisms of the plant. Pyoverdine, a yellow-green pigment produced by many fluorescent *Pseudomonads* functions as siderophore, and suppresses the pathogens (Becker & Cook 1988). Few siderophores can even chelate heavy metals and radionuclides (such as Al, Cu, Cd, In, Ga, Pb, U, and Np) and alleviate the stress imposed on plants (Neubauer *et al.* 2000).

#### **Indirect mechanisms of plant growth promotion**

PGPR effectively compete with pathogens for nutrients or niche by releasing lytic enzymes/antimicrobial compounds and by inducing systemic resistance (Podile & Kishore 2006) in plants (Fig. 1).

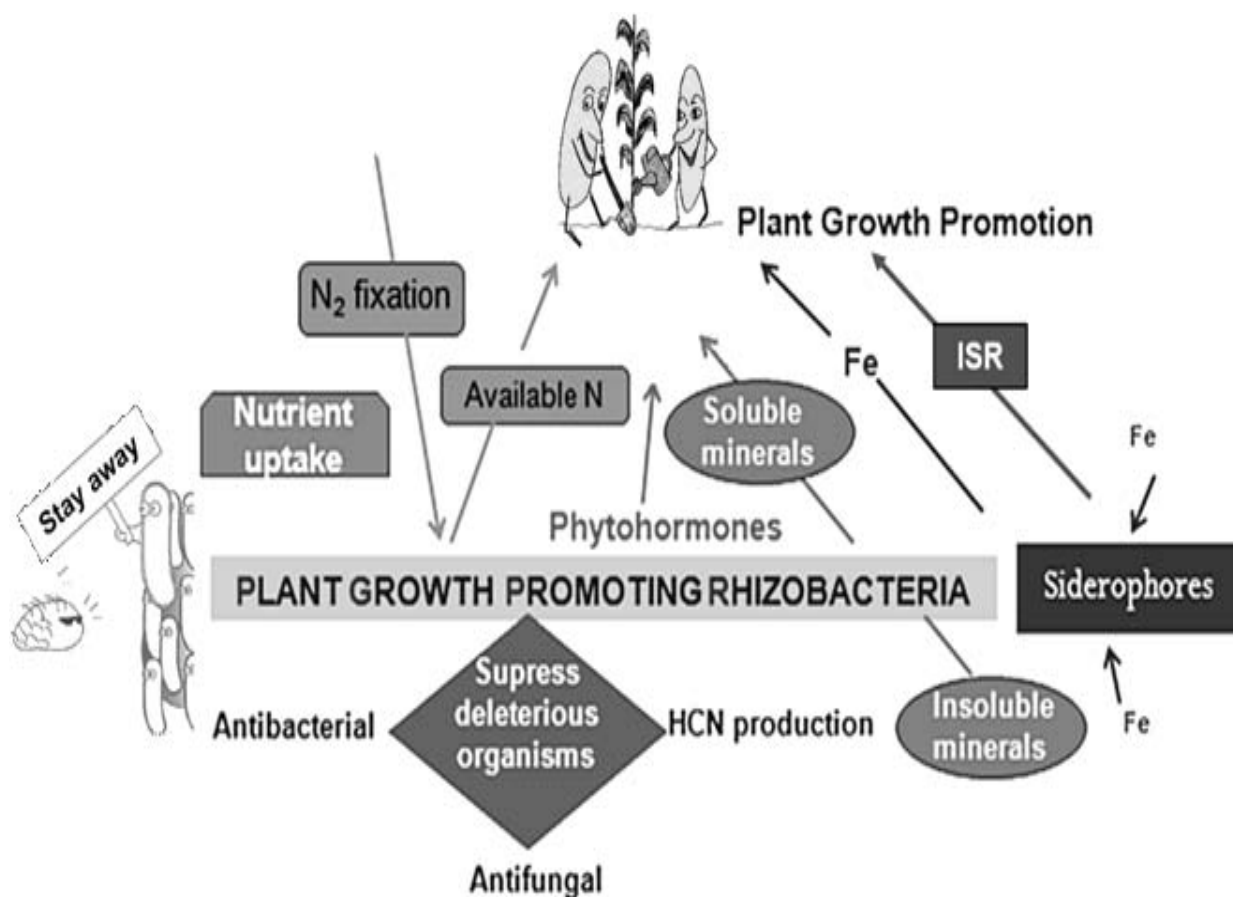
#### *Antibiosis against pathogenic microbes*

A biological association of two or more microorganisms, in which one is detrimental to

another by its antagonistic behaviour, is called as antibiosis. PGPR secrete a wide variety of antibiotics to suppress phytopathogens. These antibiotics can be antibacterial or antifungal and inhibit pathogens even at very low concentrations. Some reported antibiotics produced by *Pseudomonads* are aerugine, amphisin, azomycin, butyrolactones, cepaciamide A, ecomycins, hydrogen cyanide, 2,4-diacetylphloroglucinol, phenazine, oomycin A, pyoluteorin, tensin, tropolone, pyrrolnitrin, viscosinamide, cyclic lipopeptides, rhamnolipids, kanosamine, zwittermycin-A, pseudomonic acid, antitumor antibiotics, cepafungins, and karalicin. They are reported to have antimicrobial, antioxidant, antitumor, antiviral, antihelminthic, cytotoxic, phytotoxic activities and plant growth promotion (Goswami *et al.* 2016; Parray *et al.* 2016). Antibiotics produced by *Streptomyces*, *Bacillus*, and *Stenotrophomonas* include oligomycin A, kanosamine, zwittermycin A and xanthobaccin (Parray *et al.* 2016). The volatile hydrogen cyanide (HCN), among them, inhibits cytochrome C oxidase, an important electron transport chain enzyme and reduces the energy supply to cell. This eventually leads to death of pathogen. As the HCN is not a specific inhibitor of pathogens, it may inhibit PGPR or plant energy mechanisms as well. The phytotoxic effects of HCN in reducing plant growth is also reported in crops (Devi *et al.* 2007; Kumar *et al.* 2015). Rijavec & Lapanje (2016) proposed the phosphate regulating ability of HCN, over the biocontrol activity against pathogens, which needs further evaluation.

#### *Induced systemic resistance*

PGPR or antimicrobial compounds released by them, trigger a mild innate immune response after colonizing plants, which is referred as priming. First, microbe-associated molecular patterns of PGPR are recognized by pattern recognition receptors of plant cells. Later, the plant develops an induced systemic resistance (ISR) by producing phytoalexins, expressing PR proteins, activating mitogen-activated protein kinase, and altering cellular calcium ( $Ca^{2+}$ ) levels. This prepares the plant to fight against subsequent pathogens attack. ISR in plants is attained by activating the signalling pathways regulated by jasmonic acid or salicylic acid or ethylene. In



**Fig. 1.** Overview of plant growth promotion by PGPR: Direct and indirect mechanisms in improving the plant growth. They increase nitrogen (N), phosphate, zinc, and iron (Fe) availability and release phytohormones for improving plant growth. Antimicrobial metabolites, lytic enzymes, and siderophores secreted by PGPR prevent pathogens attack by ISR.

*Pseudomonas* spp. 'O' antigenic side chain of lipopolysaccharides, 2, 4-diacetylphloroglucinol, volatiles (like, acetoin and 2, 3-butanediol), siderophores (like pseudobactin and pseudomanine), flagella, cyclic lipopeptides, and homoserine lactones are few determinants of ISR in plants (Ryu *et al.* 2004; Gupta *et al.* 2015; Goswami *et al.* 2016). Diseases and damage caused by fungi, bacteria, viruses, nematodes and insects can be reduced by the PGPR application through activation of ISR (Ramamoorthy *et al.* 2001; Sivakumar *et al.* 2015; Sreeja *et al.* 2016; Bhai *et al.* 2017; Aswathi & Ushamalini 2017). Inoculation of *Bacillus velezensis*, *B. mojavensis*, *B. safensis*, *B. subtilis*, and *B. altitudinis* individually or in mixtures, reduced the *Heterodera glycines* (nematode) population and increased yield of soybean by activating the ISR (Xiang *et al.* 2017). *B. pumilus* SE34, *Pseudomonas fluorescens* 89B61,

and *P. putida* inoculation in tomato plants reduced the late blight (fungal) disease and *Spodoptera litura* (insect) infestation by exerting ISR (Yan *et al.* 2002; Bano & Muqarab 2017). From this, ISR can be considered as a crucial defense mechanism in plants against most of the pathogens like nematodes, insects, and fungi.

#### *Cell wall degrading enzymes*

Several PGPR show hyperparasitic activity on fungal pathogens by producing fungal cell wall degrading enzymes. As the fungal cell walls have a considerable amount of chitin and  $\beta$ -glucans as structural components, chitinases, and  $\beta$ -glucanases are considered as major mycolytic enzymes active against the number of phytopathogenic fungi (García-Cristobal *et al.* 2015; Kim *et al.* 2015). Degrading fungal cell walls

by these enzymes inhibits the fungal growth and propagation. Chitinase producing PGPR include *Bacillus* spp., *Serratia marcescens*, *Enterobacter agglomerans*, *Pseudomonas aeruginosa*, and *P. fluorescens*. Few glucanase producers are *Paenibacillus*, *B. Cepacia* and *Streptomyces* (Goswami *et al.* 2016). This is the major mechanism adapted by most of the PGPR to overcome fungal attacks in plants.

### Drawbacks of PGPR biofertilizers

Currently, a number of PGPR, with potential to enhance crop yield, are being commercialized. Various PGPR have been formulated individually or in a consortium for plant growth and defense against pathogens. Groundnut yield was significantly increased in combined application of *Rhizobium*, *Pseudomonas*, and *Bacillus* (Mathivanan *et al.* 2014). Defense against anthracnose, angular leaf spot, and wilt causing pathogens in cucumber plants was reported by Raupach & Kloepper (1998) by application of *B. subtilis*, *B. pumilus*, and *Curtobacterium flaccumfaciens*.

The main drawback in using PGPR as biofertilizers is their inconsistency and irreproducibility in their performance under field conditions. The variation in PGPR biofertilizers performance is due to environmental factors that affect their stability and growth. Eventually, this hinders their potential growth promotion in plants. About 90% of the applied biofertilizer is noted to be lost into the air while applying and not used by the plants (Vejan *et al.* 2016). The remaining population might not be optimum for colonization. Ultimately this leads to a rapid decline in the population (Arora *et al.* 2010). This might be one of the reasons for negative results in the field conditions vs. positive outcomes in the laboratory observations. Other factors affecting may be the poor expertise of farmers for inoculum application, handling, storage, and large field area for inoculation (Bashan *et al.* 2014).

### Upcoming strategies to overcome disadvantages of biofertilizers

To maximize interactions of nursery seedlings and PGPR, it is essential to determine, how they exert their positive effects on plants.

Understanding the interactions at molecular and physiological levels (Vessey 2003) with the focus on genes, proteins, and metabolites (Parray *et al.* 2016) can fill the gaps. New concepts like rhizo-engineering for pointing the exotic biomolecules responsible for the plant-microbe interactions (Gupta *et al.* 2015) and nanotechnology for the production of PGPR based nano-fertilizer for an efficient application (Vejan *et al.* 2016) can be potent alternatives. Gregorio *et al.* (2017) generated nanofibers immobilized with PGPR; *Pantoea agglomerans* and *Burkholderia caribensis*. The coating of soybean seeds with these fibers showed increased plant growth. The processes involved in the colonization of PGPR to the root system and chemical signalling involved in plant-PGPR interactions need special attention, to increase the usage of PGPR in agriculture.

### Chitosan-based PGPR biofertilizer

Chitosan is a biologically active linear polymer. It is made of D-glucosamine and N-acetyl-D-glucosamine units linked by  $\beta$ -(1,4)-glycosidic bond. Chitin, the major cellular component of fungal cell walls, insects, crustaceans acts as a precursor for generating chitosan. Chitosan is a deacetylated form of chitin. It has many applications in pharmaceuticals, biopesticides, and plant growth enhancement. Chitosan induces the synthesis of callose, lignin, defense response and phytoalexins in plants. Use of chitosan in biofertilizers to alleviate fungal diseases in crop plants is in trend. For example, crustacean chitosan and *Cunninghamella elegans* chitosan alleviated *Fusarium oxysporum* infection in cowpea plants by inducing catalase, reactive oxygen species, and peroxidases (Berger 2016). The pinewood nematode, *Bursaphelenchus xylophilus* causes severe wood damage in pine plants. Application of chitosan in soil reduced the nematode population and damage caused by them (Silva *et al.* 2014). A combined application of diazotrophic bacteria (with N, P, K, accumulation ability) and crustacean chitosan improved cowpea nodules formation, shoot biomass and yield by increasing nutrients availability (Berger *et al.* 2013). Along with agricultural applications, chitosan also had many pharmacological uses like drugs, siRNA, DNA, and proteins delivery in humans. Ippolito *et al.* (2017) studied crustacean chitosan role in



inhibition of potato pathogens *Phytophthora infestans* and *Fusarium solani*. These findings indicate the role of chitosan as an elicitor and growth promoter in biofertilizers. Chitosan amendment can add an additional benefit in preparing an efficient biofertilizer.

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