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

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 β (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 (α- and β-) 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 soyabean 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 Ca²⁺ 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⁻¹ (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 (Rajaee 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⁻¹ in rice fields. They are typically found in the rice fields, hence named as paddy organisms.
Plant root system was earlier referred as symbiotic relationship between fungi and the soil and provide to plants for their growth. The AMF capture nutrients (P, N, and S) from the soil and increase the nutrient availability and alleviate some environmental stress conditions. 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 brasileense (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 beneficial microbes for sustainable agriculture
symbiotic or free-living in the soil. They are abundant near the roots. The fraction of soil influenced by roots is called as rhizosphere (Paray 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: ectorhizosphere- 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 10¹⁰-10¹² 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, Azorarcus, 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, cocc, 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 (N≡N) 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 KI 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 (\(H_2PO_4^-\) and \(HPO_4^{2-}\)) 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 fulfill 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 (Ortíz-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 affects 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, butyrolectones, cepaciamide A, ecomycins, hydrogen cyanide, 2,4-diacetylphloroglucinol, phenazine, oomycin A, pyoluteorin, tensin, tropolone, pyrrolnitrin, viscosinamide, cyclic lipopeptides, rhamnolipids, kanosamine, zwittermycin-A, pseudomiconic 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, zwittermicin 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
**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 β-glucans as structural components, chitinases, and β-glucanases are considered as major mycolytic enzymes active against the number of phytopathogenic fungi (Garcia-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 \textit{Bacillus} spp., \textit{Serratia marcescens}, \textit{Enterobacter agglomerans}, \textit{Pseudomonas aeruginosa}, and \textit{P. fluorescens}. Few glucanase producers are \textit{Paenibacillus}, \textit{B. Cepacia} and \textit{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 \textit{Rhizobium}, \textit{Pseudomonas}, and \textit{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 \textit{B. subtilis}, \textit{B. pumilus}, and \textit{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, \textit{Pantoea agglomerans} and \textit{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 \textit{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, crustaceous chitosan and \textit{Cunninghamella elegans} chitosan alleviated \textit{Fusarium oxysporum} infection in cowpea plants by inducing catalase, reactive oxygen species, and peroxidases (Berger 2016). The pinewood nematode, \textit{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 crustaceous 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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**References**


