Seed Biopriming Through Beneficial Rhizobacteria for Mitigating Soil-Borne and Seed-Borne Diseases

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Abstract

Seed priming enables seed hydration, thereby activating its metabolism without substantial germination. It also assists in rapid germination as well as enhances resistance to both biotic and abiotic stresses. Soilborne pathogens such as *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, and *Rhizoctonia* possess major threat to crop production on a global scale. These pathogens cause diseases at the time of seed germination; hence, seed biopriming approach will be advantageous for early crop protection. Further, seed biopriming also providing greater protection by biocontrol increased adherence to seed surface. Thereby biocontrol agents will be establishing prior to pathogen infection. In this context, seed biopriming is a promising technique in comparison to seed treatment, soil application, and foliar spray, thereby providing a significant contribution to sustainable agriculture.

Keywords

Seed biopriming · PGPR · *Bioprotectant* · Plant growth promotion · Disease control

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7.1 Introduction

For enhancing the production of food crops all over the world, seeds are an essential investment and a healthy seed is a key regulator of production with both qualitative and quantitative prospects. There is an agglomeration of phytopathogens in seed as well as soil which causes various seed-borne and soilborne diseases which are imposing a serious threat to crop production and storage. Hence, there is an urgent need for management of such types of diseases as can cause re-emergence of problem. Among all types of plant diseases, soilborne diseases are considered to be more limiting than others as it directly affects the production quantity and quality of many crops and accounts for 10–20% of yield losses annually worldwide (Ray et al. 2017). In India, soilborne phytopathogenic fungi are considered as the most aggressive and destructive as they are causing more than 50% loss of economically important crops annually (Pandey et al. 2018). Several fungal genera have been identified as the major phypathogens for causing root disease in various crops. *Rhizoctonia solani*, *Sclerotinia sclerotium*, *Sclerotium rolfsii*, and *Fusarium oxysporum* are considered most notable and destructive pathogens and are responsible for causing seed rot, seedling blight, root rot, and mature plant wilt diseases with 60–70% yield loss of several economic crops. The hard resting structure sclerotia produced by these phytopathogens survive for more than 3 years in soil because all of them do not germinate or die at the same time. Therefore, the sclerotia act as inoculums as they re-germinate overtime after acquiring optimal conditions and can deteriorate an agricultural area (Pane et al. 2012; Rani 2008). Seed-borne pathogens are also continuously imparting a serious threat to crop production as they are responsible for about 10 % losses in major crops, and even management is difficult due to limited availability of effective chemicals (Chahal 2012). Various strategies have been employed to manage these diseases including cultural, chemical, and regulatory methods. In the past few decades, synthetic agrochemicals are widely used for seed treatment as a potent approach toward management of soilborne and seed-borne diseases, and commencement of systemic fungicides added further possibilities to it. However, the increasing concerns about their hazardous impact on environmental sustainability and human health initiate their reduced application in management practices. Therefore, biological control by antagonistic microorganisms emerges as a potential, non-chemical, and eco-friendly approach for providing protection to crops against various phytopathogens and is also helpful for mitigation of several plant diseases (Papavizas 1984). Now, the management of seed-borne and soilborne pathogens through seed biopriming with agriculturally important microbial antagonists is a model delivery system as it brings in the microbial inoculums to the rhizosphere. It is also a safer alternative to conventional management practices which have severely affected the environment and agroecosystem (Abhilash et al. 2016). So, sowing of a primed seed may lead to a disease-free offspring with enhanced plant growth promotion activity and decreased number of primary infection sites prone to disease dissemination. In reference, the present study describes plant growth-promoting rhizobacteria,

especially their category and mode of action, which are involved in plant growth promotion and amelioration of soilborne and seed-borne diseases.

7.2 Seed Biopriming: A Novel Concept for Seed Immunization with Beneficial Rhizobacteria

Seed treatment with PGPRs is a very old practice. Legume seed inoculation with nitrogen-fixing bacteria has a long history and enhances the legume production worldwide (Graham and Vance 2003). Regardless of encouraging results of legume seed inoculation and in vitro demonstration of the efficacy of other beneficial microorganisms, there are still very few commercially available microbial seed inoculants. Seed treatment with broad-spectrum fungicides is often essential to escape seedling establishment failure caused by various seed-borne or soilborne phytopathogens. Application of PGPR for seed biopriming to manage seed- borne and soilborne pathogens is a model delivery system as it brings in the microbial inoculum to the rhizosphere. Wide ranges of bacterial antagonists have been commercially exploited for this purpose (Nelson 2004; Berg 2009), but their applications as seed biopriming are very limited. With the time advancement, intensive researches have been done in the field of seed priming technique, and now it is being commonly used for seed immunization for better crop establishment, yield, and crop protection. Over the previous methods, this procedure of application provides a model environment to bioagents for colonization of the seed. "Soaking the seeds in a solution containing the desired microorganism followed by re-drying of the seeds that result into the start of germination process except the radicle emergence is seed biopriming" (McDonald 1999). According to Abuamsha et al. (2011), "soaking the seeds in the bacterial suspension for a pre-calculated period of time to allow the bacterial imbibition into the seed is known as biopriming." Seed soaking in bio-agent suspension resulted in activation of physiological processes in the seed. However, the emergence of plumule and radical is prevented until the seeds are sown. Seed biopriming with PGPRs has been performed in various crops including sweet corn (Callan et al. 1991), carrot (Murunde and Wainwright 2018), and tomato (Harman and Taylor 1988). Seed biopriming has been reported to facilitate the survival of bio-agents in/on seed surface, thus providing better plant growth and yield (Fig. 7.1) (Bisen et al. 2015; Singh et al. 2016; Singh 2016).

7.3 PGPR as Bioprotectant for Management of Soil-Borne and Seed-Borne Diseases

Diverse genera of bacteria are found in soil which play a key role in plant-soil-microbial interaction. On the basis of their interaction with the plant, they may be classified as beneficial, deleterious, and neutral (Dobbelaere et al. 2003). The beneficial group of bacterial population is known as plant growth-promoting

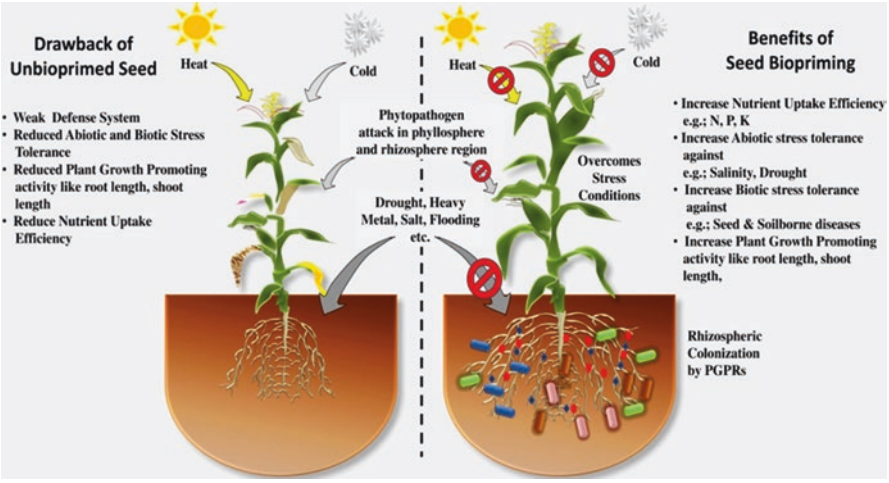

Fig. 7.1 Pictorial representation of seed biopriming effect on the crop

Table 7.1 A representative list of PGPRs on the basis of location in host

PGPRs	
Extracellular	Intracellular
Agrobacterium	*Allorhizobium*
Arthrobacter	*Bradyrhizobium*
Azotobacter	*Mesorhizobium*
Azospirillum	*Rhizobium*
Bacillus	*Frankia*
Burkholderia	*Alcaligenes faecalis*
Caulobacter	
Chromobacterium	
Erwinia	
Flavobacterium	
Micrococcus	
Pseudomonas	
Serratia	

Source: Ahemad and Kibret (2014), Bhattacharyya and Jha (2012), Ray et al. (2016)

rhizobacteria (Kloepper et al. 1989). According to their habitat location in plants, they can be categorized as extracellular (exophyte) or intracellular (endophyte) where exophyte means that they may exist in the rhizoplane (root surface), in the rhizosphere region, or between the spaces of root cortex cells (Gray and Smith 2005), whereas the intracellular bacteria are mostly located in root nodules (Table 7.1).

It has been estimated that around 2% of soil microflora comprises the population of beneficial bacteria which promotes plant growth with *Bacillus* and *Pseudomonas* as predominant species (Antoun and Kloepper 2001; Podile and Kishore 2006). These bacterial strains possess the potential to utilize as bioinoculants (BIs)/biocontrol

agents (BCAs) to protect crops from various soilborne pathogens. The prowess of PGPR as biocontrol agents or bioinoculants (biofertilizers) depends on the method of application/inoculation, concentration, physiological state, presence or absence of nutrients or adjuvants such as adhering or protective agents (Knudsen et al. 1995), host selectivity (Khan et al. 2006), and the amount of treatment (Levenfors et al. 2008). In addition, the potency of PGPRs is also affected by manufacturing protocol of BCA products (Spadaro and Gullino 2005; Fravel 2005). So, the application of these PGPRs should be done on the crops in such a way that helps to improve their efficacy in the field conditions. Utilization of these PGPRs as an alternative to synthetic agrochemical is a better choice as it protects the ecosystem from the hazardous effects of agrochemicals and maintains agro-eco-sustainability (Table 7.2).

7.4 Action Mechanism of PGPRs

PGPRs have been found effective to suppress plant diseases caused by different phytopathogens, and these antagonistic rhizobacteria also have the potential for use as bioinoculants/biofertilizers which helps to improve plant growth (Weller 2007). There are various mechanisms by which these rhizobacteria suppress the growth of phytopathogens.

7.4.1 Bioprotectant

The mechanism behind their bioprotectant nature against seed-borne and soilborne phytopathogens is through protecting the germinating seed and seedling by increasing the competition for nutrients and space in spermosphere and rhizoplane. For creating this competition, tough rhizobacteria also use various other strategies.

7.4.1.1 Production of Antibiotics

The production of antibiotic is one of the potential mechanisms of plant growth-promoting rhizobacteria against phytopathogens. A number of antibiotics have been reported to be produced by rhizobacteria to suppress pathogen growth such as phenazines, diacetyl phloroglucinol, pyocyanine, oomycin A, pyrroles, pyrrolnitrin, pyoluteorin, tropolone, and cyclic lipopeptides pseudomonads (Bender et al. 1999) and kanosamine, oligomycin A, zwittermicin A, and xanthobaccin by *Bacillus* and *Streptomyces* (Compant et al. 2005). *Pseudomonas* spp. producing antibiotic 2,4-diacetyl phloroglucinol (2,4- DAPG) and phenazine-1-carboxylic acid (PCA) have been reported to inhibit *Gaeumannomyces* graminis *var. tritici* causing take-all disease of wheat (de Souza et al. 2003; Weller 2007). Some rhizobacteria are an efficient producer of volatile compounds as hydrogen cyanide (HCN) and DAPG, which have been reported to suppress *Thielaviopsis basicola* and *Clavibacter michiganensis* sp. *michiganensis* (Sacherer et al. 1994; Lanteigne et al. 2012). Keel et al. (1992) reported that *P. fluorescens* strain CHA0 produces a number of secondary metabolites as DAPG, pyoluteorin, hydrogen cyanide (HCN),

Table 7.2 Biocontrol agents used against various seed-borne pathogens

Biocontrol agent	Phytopathogens	References
Azotobacter spp. *and Gluconacetobacter* sp. *Bacillus thuringiensis*	*Urocystis agropyri*	Wadhwa et al. (2011), Tao et al. (2014)
Bacillus megaterium	*Mycosphaerella graminicola*	Kildea et al. (2008)
Bacillus subtilis GBO3 *Bacillus pumilus* SE34	*Xanthomonas oryzae* pv. *oryzae*	Udayashankar et al. (2011), Velusamy et al. (2006)
Bacillus amyloliquefaciens	*Xanthomonas oryzae* pv. *oryzicola*	Zhang et al. (2011)
Bacillus licheniformis	*Phoma medicaginis*	Slimene et al. (2015)
Bacillus spp. SJ 5	*Fusarium* spp.	Jain et al. (2017)
Burkholderia cepacia	*Fusarium* spp.	Recep et al. (2009)
Pseudomonas fluorescence	*Helminthosporium oryzae*	Arumugam et al. (2013)
Pseudomonas chlororaphis MA 342	*Tilletia caries*	Johnsson et al. (1998)
P. chlororaphis MA 342	*Ustilago nuda*	Johnsson et al. (1998)
P. chororaphidis MA 342 *Lactobacillus acidophilus Bifidobacterium bifidus Streptococcus thermophillus*	*Tilletia tritici*	Borgen and Davanlou (2001)
Pseudomonas fluorescence	*Ustilagosegetum* var. *tritici*	Singh and Maheshwari (2001)
Pseudomonas fluorescence	*Helminthosporium oryzae*	Arumugam et al. (2013)
P. fluorescence Pseudomonas syringae pv. syringae	*Pyricularia oryzae*	Arumugam et al. (2013), Smith and Métraux (1991)
P. fluorescens PTB 9 *P. fluorescens Lysobacter antibiotics Pseudomonas* spp. *P. putida*V14i	*Xanthomonas oryzae* pv. *oryzae*	Vidhyasekaran et al. (2001), Ji et al. (2008), Rangarajan et al. (2003)
Pantoea agglomerans	*Pseudomonas syringae* pv. *syringae*	Braun-Kiewnick et al. (2000)
Pseudomonas fluorescens Bacillus subtilis (Bs16)	*Alternaria solani*	Latha et al. (2009)
P. fluorescens	*Colletotrichum lindemuthianum*	Amin et al. (2014)
Rahnella aquatilis Bacillus spp. *Rhodococcus fascians Bacillus cereus Pseudomonas aeruginosa*	*Xanthomonas axonopodis* pv. *phaseoli*	Sallam (2011), Giorgio et al. (2016), Spago et al. (2014)
Streptomyces spp.	*Drechslera maydis*	Bressan (2003)

(continued)

Table 7.2 (continued)

Biocontrol agent	Phytopathogens	References
*Stenotrophomonas maltophilia Achromobacterxylos oxidans Streptomyces globisporus*JK-1	*Magnaporthe grisea*	Etesami and Alikhani (2016), Chern et al. (2014), Joe et al. (2012), Li et al. (2011)
Streptomyces spp.	*Xanthomonas oryzae* pv. *Oryzae*	Hastuti et al. (2012)
Streptomyces spp. *Rhizobium meliloti B. subtilis* BN1 *P. fluorescens*	*Macrophomina phaseolina*	Hussain et al. (1990), Arora et al. (2001)
StrainK61 of *Streptomyces griseoviridis*	*Pyrenochaeta lycopersici*	Minuto et al. (2006)

pyoverdine, salicylic acid, and pyochelin effective against soilborne plant pathogenic fungi.

7.4.1.2 Production of Siderophore

Iron is one of the crucial elements for growth and survival in all living organisms. It is in ample amount in the Earth's crust, but most of it exists as ferric hydroxide, an insoluble form at neutral and alkaline pH. Siderophores are low molecular weight molecules that sequester ferric ion in the rhizospheric area and making them inaccessible to plant pathogens (Mehnaz 2013). Siderophore and ferric ions bind forming a siderophore-ferric ion complex, which later binds with bacterial cell surface receptors and eventually converted to ferrous ions in the cytoplasm. Different types of siderophores produced by plant growth-promoting bacteria are involved in plant growth promotion and disease suppression (Leong 1986). The diverse types of siderophores such as catechol, pyoverdine, hydroxamate, azotobactin, and anthranilic acid are produced by different plant growth-promoting rhizobacteria. The organisms having an appropriate receptor can uptake other siderophores for its own purpose, and a wide range of organisms can use a similar type of siderophore (Koster et al. 1993; Raaijmakers et al. 1995). Bacterial genera as *Pseudomonas*, *Rhizobium, Bradyrhizobium*, *Bacillus, Burkholderia*, *Aeromonas*, *Streptomyces,* and *Serratia* have been reported to exhibit siderophore production (Kufner et al. 2008; Sujatha and Ammani 2013).

7.4.1.3 Production of Hydrolytic Enzymes

Production of a lytic enzyme is another potential mechanism used by plant growth-promoting bacteria to combat pathogen attack. The lytic enzymes as β-glucanase, chitinases, lipases, dehydrogenase, proteases, and phosphatases manifest hyperparasitic activity against attacking pathogen (Joshi et al. 2012; Hayat et al. 2010). Plant growth-promoting rhizobacteria mediated via these enzymes have been reported to protect the plant from pathogens as *Sclerotium rolfsii, Botrytis cinerea, Rhizoctonia solani, Fusarium oxysporum, Pythium ultimum*, *and Phytophthora* spp. The gene encoding chitinase enzyme from *Serratia marcescens* was cloned and

transferred to *E. coli.* The chitinase thus obtained exhibited chitinolytic activity against *Sclerotium rolfsii* and *Rhizoctonia solani* (Chet et al. 1990, 1993).

7.4.1.4 Induction of ISR

Application of biocontrol agents elicits an enhanced defense in plant system against subsequent pathogen challenges (Avis et al. 2008). ISR primed plant responds rapidly to attack by different pathogens and does not involve direct interaction between plant growth-promoting rhizobacteria and pathogen. It is instigated by prior inoculation of the host by incompatible or avirulent forms of a pathogen and plant growth-promoting bacteria against subsequent inoculation by the virulent pathogens. Induced systemic resistance involves jasmonic acid and ethylene as a signaling molecule and stimulates defense against fungal, bacterial, viral, and nematode diseases (Naznin et al. 2012; Glick 2012). Seed biopriming with plant growth-promoting rhizobacteria triggers a broad-spectrum systemic resistance against a large number of pathogens. Bacterial components such as flagella, lipopolysaccharides (LPS), siderophores, homoserine lactones, 2,4-diacetyl phloroglucinol, cyclic lipopeptides, and volatiles as 2,3-butanediol and acetoin can induce systemic resistance in the plant (Doornbos et al. 2012).

7.4.2 Plant Growth Promotion

7.4.2.1 Modulation of Phytohormone Production

Plant growth-promoting rhizobacteria have the ability to produce phytohormones as auxins, gibberellins, cytokinins, and ethylene which have a key role in plant growth and development (Davies 2010; Arora et al. 2013). Plant under environmental stress shows alteration in phytohormone level. Plant growth-promoting bacteria have the ability to produce phytohormones and thereby alter plant response under stress condition (Glick et al. 2007; Salamone et al. 2005). The cytokinins and gibberellins have been reported to be produced by PGPR and have a stimulatory effect on plant growth as cytokinins produced by them are in lower concentration compared to pathogens which have an inhibitory effect. *Pseudomonas*, *Rhizobium*, *Bacillus*, *Azospirillum*, *Enterobacter*, and *Acinetobacter* have been reported to produce auxin and ethylene whereas *Azotobacter* sp., *Pseudomonas* sp., *Rhizobium* sp., *Bacillus* sp., *Rhodospirillum rubrum*, and *Pantoea agglomerans* produce cytokinins and gibberellins (Kang et al. 2010; Shilev 2013). These PGPRs enhance mineral, nutrient, and water uptake by the proliferation of plant roots and root hairs (Arora et al. 2013).

Indole acetic acid (IAA) is produced by about 80% of rhizobacteria, and it regulates cell division and differentiation, stimulates seed and tuber germination, enhances rate of xylem and root development, initiates lateral and adventitious root formation, affects photosynthesis and pigment formation, and regulates responses to gravity and light, biosynthesis of metabolites, and resistance under stress (Spaepen and Vanderleyden 2011). Ethylene affects plant growth and development

by promoting root initiation, fruit ripening, leaf abscission, and seed germination and inhibits root elongation (Glick et al. 2007).

7.4.3 Increase Nutrition Uptake

7.4.3.1 Nitrogen Fixation

Despite the nitrogen being 78% of all gases in the atmosphere, it remains unaccessible to plants. Out of all the organisms on Earth, plant growth-promoting rhizobacteria are gifted with the ability to fix atmospheric nitrogen, making them available to plants. The PGPR fixes atmospheric nitrogen by two mechanisms: symbiotic and non-symbiotic. The symbiotic nitrogen-fixing bacteria remain in close association with plant root and enters the root, forming nodules. The symbiotic plant growth-promoting bacteria include *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, and *Mesorhizobium* with leguminous plants and *Frankia* with non-leguminous trees and shrubs (Zahran 2001). The non-symbiotic nitrogen-fixing genera include *Azotobacter*, *Azospirillum*, *Acetobacter*, *Burkholderia*, *Enterobacter*, *Pseudomonas Gluconacetobacter,* and cyanobacteria as *Anabaena and Nostoc* (Vessey 2003; Bhattacharyya and Jha 2012). Both symbiotic and free-living nitrogen fixers contain nif genes for nitrogen fixation. The application of PGPR on crop provides overall management of diseases, promotes growth, strengthens defense system of plants, and maintains soil nitrogen level (Reed et al. 2011; Gupta et al. 2015).

7.4.3.2 Phosphate Solubilization

Phosphorus is the second most essential element after nitrogen to plants. It plays a key role in almost all metabolic processes like photosynthesis, respiration, energy transfer, signal transduction, and macromolecular biosynthesis (Khan et al. 2010). Phosphorus is present in an abundant amount in the soil as an insoluble and immobilized form which cannot be utilized by plants (Pandey and Maheshwari 2007). The plant growth-promoting rhizobacteria release phosphorus from complex insoluble, immobilized to soluble form as *the monobasic* (H_2PO_4) and the diabasic (HPO_4^{2-}) ions. Phosphate-solubilizing PGPR is included in the genera *Bacillus*, *Pseudomonas*, *Rhizobium*, *Beijerinckia*, *Burkholderia*, *Arthrobacter*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhodococcus*, *Microbacterium*, and *Serratia* (Bhattacharyya and Jha 2012).

7.4.3.3 Potassium Solubilization

Potassium ranks third in essentiality criteria after nitrogen and phosphorus. About 90% of potassium exists in the soil as insoluble rocks and silicate minerals which are solubilized through secretion of organic acids (Parmar and Sindhu, 2013). Potassium-solubilizing plant growth-promoting rhizobacteria such as *Bacillus edaphic*, *Acidithiobacillus ferrooxidans*, *Burkholderia*, *Pseudomonas*, *Bacillus mucilaginosus,* and *Paenibacillus* sp. solubilize potassium making them available to plants (Liu et al. 2012; Gupta et al. 2015). Inadequate potassium leads to retarded root growth, smaller seeds, and lesser yield. Application of potassium-solubilizing

PGPR as biofertilizer is an eco-friendly approach to combat potassium deficiency by avoiding the use of excessive agrochemicals (Banerjee et al. 2006).

7.5 Future Prospects of PGPR Incorporation in Seed and Soil

Lack of sufficient management strategies, limited availability of biopesticides, and outdated chemicals are major constraints for the management of seed-borne and soil-borne pathogens (Agarwal and Sinclair 1996). Utilization of AIMs for the management of these problems is a safer alternative rather than chemical management practices for the sustainability of our environment and agroecosystem. PGPR is an eminent component of the biopesticide industry to improve agricultural production in the long run. In the last few decades, a large number of PGPRs genera have been screened, characterized, and identified, and their application has been boosted manifold. Globally, approximately 90% of bacteria-based products are available (Nion and Toyota 2015), and in India, we have 121 registered bacterial products (http://cibrc.nic.in/bpr.doc). But still, the use of PGPR is, to a limited extent, on field level even though its efficacy has been proved in laboratory, greenhouse, and field conditions. Seed biopriming provides an opportunity for the seed industry to provide better-quality seeds to growers to mitigate seed-borne and soilborne diseases in a safer way. Future researches need to be directed toward seed-microbe interaction at the time of seed biopriming, for standardization of products and development of a universal delivery system for seed biopriming. Biotechnological and molecular approaches can be directed toward better understanding of microbe interaction with seed and ideal condition for storage and use of primed seed. Further, laws regarding production, commercialization, and application of bacterial products for seed biopriming need to be framed for popularizing such products among farmers.

7.6 Conclusion

Plant growth-promoting bacteria, being multitasking with the ability of plant growth promotion, disease suppression, bioremediation, and biofertilization, is expected to replace chemical fertilizers, artificial growth regulators, and chemical pesticides completely in the near future. With the increasing problem of chemical residue accumulation, biomagnifications and other environmental issues have urged the need to move toward a sustainable agriculture. Future researches need to be directed toward exploring competent PGPR strains with properties to survive under diverse agroecological conditions as extreme temperatures, salinity, drought, etc. Apart from laboratory and greenhouse application, there is an urgent need to implement it on large scale, and researches should be carried upon major constraints in the field application of PGPR. New approaches need to be developed for enhancing storage, growth, formulation, shipping, and application of PGPR (Glick 2012). Scientists need to develop more efficacious bacterial strains to fulfill the above needs by

screening or genetic engineering approaches as well as convince the public and regulatory authorities for its safety toward humans and the environment.

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