Management of Plant Diseases by PGPR-Mediated Induced Resistance with Special Reference to Tea and Rice Crops

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Abstract

Among the biotic stresses, plant pathogens can reduce yield crop which affected potential loss to crop productivity. Plant growth-promoting rhizobacteria (PGPR) can help plants to be resistant against biotic stress via direct antagonism to pathogens or by induction of systemic resistance to pathogens. The presence of high levels of nutrients exuded from various roots of most plants can support bacterial growth and metabolism as well as maintain health of the plant in the growth process. PGPR promote plant growth due to their abilities in phytohormone production, nitrogen fixation, and phosphorus solubilization; produce several substances which are related to pathogen control, i.e., exhibiting competition with plant pathogens, synthesis of antibiotics, antifungal metabolites and defense enzymes, and secretion of iron-chelating siderophores; and trigger induced systemic resistance (ISR) via methyl jasmonate and methyl salicylate in plants. The ISR resembles pathogen-induced systemic acquired resistance (SAR) through the salicylic acid-dependent SAR pathway under conditions where the inducing bacteria and the challenging pathogen remain spatially separated. The use of PGPR combinations of different mechanisms of action, i.e., induced resistance and antagonistic PGPR, might be useful in formulating inoculants leading to a more efficient use for biological control strategies to improve crop productivity. Many PGPR have been isolated from the tissues of many plants, and various species of bacteria, i.e., *Azotobacter*, *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Serratia*, have been reported to control several diseases and enhance plant growth. PGPR belonging to the genera *Pseudomonas* and *Bacillus* are also well known for their

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antagonistic effects and their ability to trigger ISR. An increasingly successful study to reduce disease severity is the use of bacteria, namely, *Bacillus subtilis*, *P. fluorescens*, *Serratia*, and the fungus *Trichoderma*. Tea and rice plants are cultivated in Indonesia predominantly in Java and Sumatra islands. Major constraints of cultivation include low fertility of soils, poor input management, low germination, and high susceptibility to the diseases. The strategies employed by PGPR provide promising approaches to alter agricultural crops and plantation practices toward sustainable environmental development. Research has been conducted to know the effect of PGPR on tea plant growth that can work optimally as a biological fertilizer and plant-induced resistance to suppress blister blight (*Exobasidium vexans* Massee), a major disease in tea plantation that can decrease yield loss up to 50%. Individual PGPR strains for in vitro broad-spectrum pathogen suppression and production of several physiological/biochemical activities related to plant growth promotion have been screened. Numerous bacterial isolates have been found to function both as biofertilizers and biological control agents, namely, *Chryseobacterium* sp. AzII-1, *Acinetobacter* sp., *Alcaligenes* sp. E5, *Bacillus* E65, and *Burkholderia* E76. Study about synergism among bacteria has been carried out in the laboratory test using four combinations, i.e., (a) *Chryseobacterium* sp. AzII-1 + *Acinetobacter* sp., (b) *Chryseobacterium* sp. AzII-1 + *Alcaligenes* sp. E5, (c) *Chryseobacterium* sp. AzII-1 + *Bacillus* E65, and (d) *Chryseobacterium* sp. AzII-1 + *Burkholderia* E76. All bacterial combinations had a synergistic effect. It was shown that the bacterial population was not significantly different with the average of the total bacterial population (4.62×10^8 CFU/ml). The effect of bacterial combinations to blister blight and plant growth under a tea nursery trial revealed that combination of *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25% could increase the growth of tea plant and suppress the intensity of blister blight up to 1.27%. The disease intensity of blister blight decreased in all treatments under field trial, while the *Acinetobacter* sp. treatment in tea shoots was 17.26% higher than the control. PGPR have also been isolated from cultivated rice. *Serratia* SKM, *Burkholderia* E76, and *Bacillus* E65 have the potential for controlling rice diseases and induce plant growth promotion. Under in vitro antagonistic assay, it was shown that these isolates could suppress effectively the growth of rice pathogens *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial blight (BB). Kaolin formulation of these three isolates was evaluated as a foliar application on rice. PGPR application under experimental plots resulted in enhancement of rice growth and yield, with the yield increment on cv. Sintanur being 12.8 percent higher compared with control (cv. Ciherang). Based on PGPR application technology which is demonstrated in farmers' plots, the severity of BB disease was reduced to 76.8 percent compared with the untreated plot. The farmers were convinced with the beneficial effects of PGPR on both plant growth and yield and reduction of BB disease incidence. PGPR technologies have the potential to reduce agrochemical application. They can also be exploited as low in input and environmentally friendly for sustainable plant management. PGPR is highly diverse, and in this review, we focus on PGPR in plant growth promotion, as well as understanding the role of PGPR in crop protection.

4.1   Introduction

Agricultural crop production is strongly exposed to many stresses of biotic and abiotic factors, leading to yield loss of crops. Globally, inappropriate fertilizer and high severity of plant disease factors may reduce yield that threatens food security. To keep the stability of crop production, the current strategy is based primarily upon chemical compounds as reliable methods. Chemical fertilizers are used to provide sufficient nutrients for optimizing crop yields. However, the reliance on the use of synthetic inorganic fertilizers and pesticides often creates the pathogen resistance to chemicals, environmental pollution, and deleterious nontarget effects on humans and animals (Waard et al. 1993). Therefore, there is a need to develop alternative control approaches for crop protection. The interest in the use of plant growth-promoting rhizobacteria (PGPR) that enhance plant health has increased and gained interest worldwide due to public concern for sustainable agriculture because they can promote plant growth as well as provide biological control (BC) of plant diseases (Kloepper and Schroth 1978; Schnider et al. 1994; Emmert and Handelsman 1999; Beneduzi et al. 2012).

The use of organic biofertilizers or biopesticides containing PGPR isolates is an alternative strategy to reduce chemical supplements (Subba-Rao 1993; Banerjee et al. 2005; Chandler et al. 2011; Saharan and Nehra 2011; Amar et al. 2013). PGPR agents, promote plant growth by several mechanisms, i.e., alteration in the rhizosphere microbial community structure, nitrogen fixation (Bhattacharjee et al. 2008), phosphate solubilization, plant growth regulation (IAA, gibberellins, and cytokinins) (Gilbertson et al. 2007; Setyowati et al. 2017), secretion of iron-chelating siderophore, production of volatile organic compounds (VOC), and exerting deleterious effects on other microorganisms (Kloepper et al. 1980; Glick 1995; Verma et al. 2011; Labuschagne et al. 2011; Liu et al. 2013).

The rhizosphere is populated by a diverse range of PGPR (Schroth and Hancock 1982). This habitat is rich in nutrients which provide organic carbon sources due to the accumulation of a variety of plant exudates such as simple/complex sugars (glucose, xylose, maltose, and sucrose), primary and secondary compounds including amino acids (aspartic acid, glutamic acid, isoleucine, and leucine), organic acids (citric acid, malic acid, lactic acid, and succinic acid), phenolic acids, flavonoids, enzymes, fatty acids, nucleotides, tannins, steroids, terpenoids, and alkaloids (Campbell et al. 1990; Kaitaniemi and Honkanen 1996; Walker et al. 2003; de Weert et al. 2004; Rudrappa et al. 2008; Gray and Smith 2005).

On the basis of plant growth effects, plant-associated bacteria can be classified into beneficial, deleterious, and neutral groups (Dobbelaere et al. 2003). The first step for PGPR beneficial effects is the successful colonization on the root (Choudhary and Johri 2009; Piromyou et al. 2011). In the rhizosphere population, the bacteria that promote plant growth were found to be about 1–2% (Antoun and Kloepper 2001). A number of bacteria are found around the roots of plants, which is generally tenfold higher

than that in the bulk soil (Weller and Thomashow 1994). The cultivable rhizosphere bacteria were detected in soil to be approximately 10^7–10^9 CFU/g compared with rhizoplane bacteria which was approximately 10^5–10^7 CFU/g (Benizri et al. 2001; Ugoji et al. 2005). Thus, an important aspect of colonization has been the ability to compete with indigenous microorganisms already present in the soil and rhizosphere of the inoculated plant (Schroth and Hancock 1982; Waard et al. 1993). The efficient bacterial root colonization was reported by *P. putida* on potato roots and by *P. fluorescens* WCS365 on tomato root tips (de Weger et al. 1989; Dekkers et al. 1998).

PGPR have improved soil quality via soil remediation, increasing the availability of nutrients for PGPR, and eliminating plant pathogens. The beneficial effects of PGPR on plants usually are separated into two categories, i.e., biocontrol of plant disease and growth promotion, which have a close relationship with each other (Mariano and Kloepper 2000). The beneficial PGPR can reduce the incidence or severity of plant diseases as BC agents are termed as microbial antagonism, whereas those exhibiting antagonistic activity toward a pathogen are termed as antagonists (Beattie 2006). As agents for BC, PGPR exhibit two major mechanisms, i.e., (a) direct mode antagonism in which the PGPR produce metabolites that directly affect the pathogen (antibiosis, competition, and hyperparasitism) (Beneduzi et al. 2012) and (b) indirect mode (induced systemic resistance) in which the PGPR triggers plant resistance against the pathogen (Glick 1995). PGPR can produce a wide variety of compounds with antimicrobial activity used as defense systems. The following PGPR environment and bacterial antagonistic activities can be highlighted: (a) synthesis of hydrolytic enzymes, such as chitinases, glucanases, proteases, and lipases that can lyse pathogenic fungal cells (Maksimov et al. 2011); (b) competition for nutrients and suitable colonization of niches at the root surface (Döbereiner 1992; Patten and Glick 2002; Kamilova et al. 2005); (c) regulation of plant ethylene levels through the ACC deaminase enzyme, which can act to modulate the level of ethylene in a plant in response to stress imposed by the infection (Glick et al. 2007; Van Loon 2007); and (d) production of siderophores, bacteriocins, and broad-spectrum antibiotics as antagonistic activities (Baker and Cook 1982; Riley and Wertz 2002). The ability of PGPR to produce siderophore metabolites contributing to antibiosis has been deeply investigated. The uptake of ferric ion via siderophore is largely used by pathogenic and nonpathogenic microorganisms from the environments. Siderophores, bacteriocins, and antibiotics are three of the most effective and well-known mechanisms of antagonist to prevent phytopathogenic proliferation (Maksimov et al. 2011).

The recent global need for healthier foods with less contamination from chemical residues, as well as a great concern for the preservation of the environment, has been increased; however, few BC agents are currently available in the market. An attempt to isolate PGPR organisms from the rhizospheres of crop plants and the compost is quite well-conducted worldwide. To support sustainable agriculture, the interaction between PGPR and plants has been exploited commercially. Applications of these associations have been investigated in many crops, such as soy, wheat, oat, maize, potatoes, barley, peas, canola, tomatoes, lentils, and cucumber (Khalid et al. 2004; Gray and Smith 2005; Podile and Kishore 2006).

Bacteria of diverse genera have been identified as PGPR, of which *Bacillus* spp. and *Pseudomonas* spp. are important and predominant genera which are aggressive to colonize the rhizosphere of various crops and have a broad spectrum of antagonistic activity to many pathogens (Podile and Kishore 2006). Use of antagonistic PGPR strains has been demonstrated to many plant pathogens, e.g., *Fusarium* spp., *Pseudomonas* spp., *Pythium* spp., *Rhizoctonia solani*, and *Xanthomonas* spp. (Yuan et al. 2012). A screening strategy to select root colonization mutants of *B. amyloliquefaciens* strain FZB42 was reported using green fluorescent protein-tagged wild type and mutants (Dietel et al. 2013). A BC strategy on postharvest diseases in apple has been carried out by soaking treatment with *B. amyloliquefaciens* strain 9001 (Li et al. 2015).

PGPR are known to affect disease reduction and plant growth; however, some strains that are effective in vitro or in the greenhouse may not be effective under field conditions. Various environmental factors may affect PGPR strains' growth and change their effects on the plant. PGPR strains that have broad-spectrum BC activity and multiple plant growth-promoting traits are a possible approach for allowing their adaptation to a complicated environment. Most BC studies evaluate a single PGPR strain against a single-target pathogen (Zhang et al. 2010). However, under environmental conditions, a single PGPR strain as BC may suppress an only narrow range of pathogens and exhibit inconsistent performance. Therefore, mixtures of PGPR have been used to manage multiple plant diseases that often occur in the field (Domenech et al. 2006; Jetiyanon and Kloepper 2002).

This paper overviews value involved in the PGPR BC of pathogens in the field and will hopefully stimulate further investigation into advanced plant disease management as well as minimize the use of chemicals, which is essential to overcome environmental and health concerns. In addition, several recent technologies of bacterial determinants important for BC were also briefly reviewed. The review paper was organized as follows: (1) PGPR colonization, (2) PGPR and plant growth promotion, (3) PGPR as BC agent and their mechanism, (4) defense mechanisms of ISR mediated by PGPR, and (5) current research toward the development of BC agent capacity in understanding the microbial determinants of BC and plant responses. It also mentioned here an example of the results of our studies in the management of plant diseases using rhizosphere microbes, with special reference to tea and rice crops.

4.2   PGPR Colonize Plant

The influence of PGPR to plant growth and disease reduction was made by direct or indirect mechanisms; however, the successful first step leading to beneficial effects is colonization of the root (Choudhary and Johri 2009; Piromyou et al. 2011). Therefore, to improve the survival and competition of inoculated strains, a deep understanding of all steps involved in the root colonization by PGPR is required (Kokalis-Burelle et al. 2005). The colonization process by bacteria in seeds or plant parts is an active process whereby bacteria can survive and multiply in the region surrounding the seed or they attach to the root surfaces (Kloepper and Beauchamp 1992). Several PGPR colonizes the rhizosphere and rhizoplane. They also act as endophytes which spread

inside the plant and colonize internal root and stem tissues, leaves, flowers, and fruits (Hallmann 2001; Probanza et al. 2001; Hardoim et al. 2008). Root colonization by the beneficial microbe is a process which is required for all mechanisms of BC. Using plate counting, the efficiency of bacterium colonization after 15 days of plant growth was found in a range of 1.8×10^3 CFU/g on the root of the inoculated plant, while no bacterial colonies were recovered from uninoculated plants (Lugtenberg et al. 2001).

A variety of bacterial traits, such as motility, chemotaxis to seed and root exudates, production of pili or fimbriae, production of specific cell surface components, capacity to use specific components of root exudates and protein secretion, and quorum sensing, contribute to the colonization process (Lugtenberg et al. 2001; Barriuso et al. 2008; Dietel et al. 2013; Dutta and Podile 2010). PGPR move from the rhizosphere to root surfaces guided by chemotaxis and facilitated by flagella (Compant et al. 2010). Chemotaxis is an important competitive colonization trait. Mutants of *P. fluorescens* defective in flagella-driven chemotaxis but retaining motility exhibited strongly reduced root colonization. Chemotaxis assays using *P. fluorescens* WCS365 showed that amino acids (L-leucine) and organic acids are good attractants, whereas sugars have no such activity. Based on the concentrations estimated to be present in the rhizosphere, citric acid and malic acid are suggested as the major attractants during BC process (De Weert et al. 2002). The BC agent such as strain *P. chlororaphis* PCL1391 is attracted to the *Forl* hyphae by chemotaxis toward fusaric acid (FA) secreted by *Forl* (De Weert et al. 2004). The bacterial cells moved toward the fungus and kill fungal hyphae by secreting antifungal metabolite phenazine-1-carboxamide (PCN). The over present of FA will inhibits the synthesis of *N*-AHL that is required for PCN synthesis; hence, further antibiotic synthesis is inhibited. Some *Fusarium* strains have been shown to deacetylate the antibiotic 2,4-diacetyl-phloroglucinol (DAPG) to the mono-acetyl form, thereby inactivating (detoxification) the antibiotic. Some *Botrytis* strains are resistant toward phenazine because they have an active efflux pump of the antibiotic which keeps the intracellular phenazine concentration lower.

4.3   PGPR and Plant Growth Promotion

PGPR have been shown to colonize plant roots and directly enhance plant growth by a variety of mechanisms, such as nitrogen fixation, solubilization of mineral phosphate, secretion of plant hormones, and environmental stress relief (Vessey 2003; Antoun and Prevost 2006; Lugtenberg and Kamilova 2009). PGPR of different bacterial species can solubilize insoluble inorganic phosphate compounds such as dicalcium phosphate, tricalcium phosphate, rock phosphate, and hydroxyapatite for plant uptake (Nautiyal et al. 2000). Biofertilizer products containing living microorganisms colonize the rhizosphere of plants subsequently increasing the supply or availability of primary nutrients and providing a growth stimulus to the target crop. *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a produced volatile organic compound (VOC) 3-hydroxy-2-butanone (acetoin) and 2,3-butanediol that could promote significant plant growth promotion on *Arabidopsis* (Bhattacharjee and Dey 2014).

4.3.1 Nitrogen Fixation

The improvement of soil fertility is an essential strategy for increasing agriculture yield. PGPR present in the rhizosphere, rhizoplane, and plant tissues have the capacity to fix N and increase the availability/solubilization of nutrients in the rhizosphere (Rodriguez and Fraga 1999; Vessey 2003; Adesemoye et al. 2010). Nitrogen (N) is the most vital nutrient for plant growth since it is required for biosynthesis of essential molecules such as amino acids and nucleic acids (Hewitt and Smith 1974; Wetzel and Likens 2000). Although approximately 78% of the atmosphere is N in the form of N2, it cannot be directly used by any organism (Delwiche 1970). The N-fixing microorganisms convert nitrogen gas (N2) from the atmosphere into the plant utilizable form through the action of the nitrogenase enzymatic complex during N fixation (Kim and Rees 1994).

Microorganisms such as *Azospirillum*, *Cyanobacteria*, *Azoarcus*, *Azotobacter*, and *Acetobacter diazotrophicus* are examples of symbiotic nitrogen-fixing forms which can develop soil fertility by biological N fixation (Okon and Labandera Gonzalez 1994; Graham et al. 1998; Bhattacharjee et al. 2008). Two groups of N-fixing microorganisms that are symbiotic with legumes and induce the formation of nodules have been extensively studied, i.e., symbiotic N2-fixing bacteria *Rhizobium* (Zahran 2001) and *Bradyrhizobium* (Sánchez et al. 2011; Giraud et al. 2013). The nonsymbiotic N2-fixing bacteria consist of genera *Azospirillum* (Khammas et al. 1989; Fibach-Paldi et al. 2012), *Acetobacter* (James et al. 1994), *Bacillus* (Ding et al. 2005), and *Pseudomonas* (Yamanaka et al. 2005).

4.3.2 Phosphate Solubilization

In agricultural soils, phosphorus (P) is an essential macronutrient for plant growth and exists largely in unavailable forms for plants due to its insolubility. Phosphate-solubilizing bacteria exist in the rhizosphere, where they produce organic acids for solubilizing the inorganic mineral P (Gaur 1990; Bolan et al. 1994) or enzymes such as phytases which release soluble phosphorus from organic compounds of soil (Hayes et al. 2000). These processes facilitate the conversion of insoluble forms of P to be available for the plants (Rodriguez and Fraga 1999).

The most common phosphate-solubilizing bacteria belong to the genera *Azotobacter* (Kumar et al. 2001), *Pseudomonas* (Selvakumar et al. 2009), and *Rhizobium* (Sridevi and Mallaiah 2009), which can enhance plant P uptake (Yu et al. 2012). A mixture of PGPR strains *B. amyloliquefaciens* IN937a and *B. pumilus* T4 supplemented with 75% of the recommended fertilizer was equivalent to N and P nutrient uptake to the full fertilizer rate (Adesemoye et al. 2009). *Bacillus* sp., *Klebsiella oxytoca*, and *P. nitroreducens* were capable of dissolving phosphate with a phosphate solubility index range from 2.1 to 4.6 and able to stimulate the corn seed germination (Setyowati et al. 2017).

4.3.3 Phytohormones

Some PGPR strains produce phytohormones such as auxins, cytokinins, and gibberellins that stimulate plant growth (García de Salamone et al. 2001; Bottini et al. 2004; Khalid et al. 2004). The plant hormones (indole-3-acetic acid (IAA), gibberellins, and cytokinins) are known to be involved in root initiation, cell division, and cell enlargement (Bottini et al. 2004). Production of IAA by PGPR has been recognized as a mode of action on the promotion of plant growth (Etesami et al. 2009). IAA-producing PGPR can increase root growth and root length, resulting in a greater root surface area which enables the plant to access more nutrients from the soil (Patten and Glick 2002; Gilbertson et al. 2007). The corn rhizosphere was dominated by bacilliform-shaped Gram-positive bacteria capable of producing IAA in a range from 4.83 to 125.84 ppm (Setyowati et al. 2017). *P. fluorescens* which were isolated from the rhizosphere of soybean can produce cytokinins (De Salamone et al. 2006).

The IAA phytohormone production values among isolate bacteria from rice rhizosphere ranged from 6.632 to 50.053 mg/L with the highest IAA production shown by isolate 6KJ which was followed by 4 PB (41.807 mg/L). The three potential isolates belonged to *B. aryabhattai* 6KJ, belonging to *B. cibi* 4 PB and *B. marisflavi* 2 KB. Bacterial IAA increased rice seed vigour significantly compared to control. However, bacterial inoculation with different concentrations of IAA did not significantly affect the growth of rice plants (Lestari et al. 2015).

PGPR strains produce growth hormones containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase that have shown protection against stress via increased growth (Grichko and Glick 2001; Shaharoona et al. 2006; Nadeem et al. 2009; Zahir et al. 2008; Zhang et al. 2008). PGPR that produce ACC deaminase can hydrolyze ACC (the immediate precursor of ethylene) to alpha-ketoglutarate and ammonia, to promote plant growth (Mattoo and Suttle 1991; Saleem et al. 2007). Ethylene is an important phytohormone, but overproduction of ethylene under stressful conditions can result in the inhibition of plant growth or even plant death, especially for seedlings (Beyer 1976; Abeles et al. 1992).

4.4   PGPR as a BC Agent and Their Mechanisms

PGPR influence the plants' growth, yield, and nutrient uptake, as well as exhibit BC of plant disease (Kloepper and Schroth 1978; Udayashankar et al. 2011). The two main genera of PGPR strains include fluorescent of *Pseudomonas* spp., *Bacillus* spp., and Gram-positive spore-forming bacteria (Figueiredo et al. 2011). Although the preponderance of most PGPR studies has been reported to use *Pseudomonas* sp., most commercially available PGPR are bacilli because this species has dormant endospores that are tolerant to heat, desiccation, UV irradiation, and organic solvents (Brumm et al. 1991; Gates et al. 2010).

PGPR as a BC agent that protects plants exhibit several mechanisms, which can be grouped into two general mechanisms. The first is antagonism (antibiosis, competition for nutrients and niches, predation and parasitism, and inhibition of fungal

spore germination) in which the PGPR strain exerts its primary and direct action against the pathogen via antibiosis or competition. Antagonism is defined as actively expressed opposition and includes antibiosis, competition, and parasitism (Cook and Baker 1983). The basis of antagonism as a BC mechanism of PGPR has been extensively studied (Dowling and O'Gara 1994; Whipps 2001; Lugtenberg and Kamilova 2009; Govindasamy et al. 2011). Antibiosis appears to be the main mechanism by which most PGPR strains with BC activity operate (Fernando et al. 2006; El Meleigi et al. 2014). A wide variety of PGPR metabolites, including antibiotics, siderophores, and cell wall-degrading enzymes, are involved in BC (Fernando et al. 2006; Sayyed et al. 2013; Jha and Subramanian 2014). Among these metabolites, antibiotics have been extensively studied (Govindasamy et al. 2011). Numerous siderophores have been identified, while other molecules such as bacteriocins are also used for microbial defense system purposes.

Another mechanism is the indirect mode ISR in which PGPR trigger the plant resistance to the pathogen (Compant et al. 2005; Kloepper et al. 2004). Microbes acting through ISR (i.e., some strains of *Bacillus*, *Pseudomonas*, and *Trichoderma*) colonize the root where they send signals to the plant which prime the plant into a stage in which it quickly reacts on the attack by a pathogen. Individual components shown to be able to induce ISR are flagella, lipopolysaccharides, *N*-acyl homoserine lactones, siderophores, antibiotics (phloroglucinol and surfactin), and volatiles such as 2,3-butanediol produced by *Bacillus* spp. (Ryu et al. 2004). Signaling is systemic to protect all plant parts. Moreover, signaling is dependent on the plant hormones jasmonate and ethylene. ISR can protect against a variety of pathogens such as bacteria, fungi, and viruses and even insects (Van Wees et al. 2008). *P. fluorescens* WCS365 inhibits the germination of spores of the *Fusarium* fungus (Kamilova et al. 2008). Besides siderophore production, the BC abilities of *Pseudomonas* strains essentially depend on aggressive root colonization, ISR in the plant, and production of antifungal antibiotics (Haas and Keel 2003).

It has advantages to use more than one mechanism to suppress diseases. Strains acting through predation and parasitism mechanism can produce enzymes (such as chitinase, cellulase, β-1,3-glucanase, and protease) which lyse the fungal cell wall. This mechanism has the advantages that it can act without the action of antibiotics, which makes the BC agent safer than strains acting through antibiosis. Pliego et al. (2007) isolated 37 strains of BC agents which are not only good competitors but also produce antibiotics. Some strains can use a variety of mechanisms. For example, *P. fluorescens* WCS365 is an enhanced root colonizer and can also use ISR and inhibition of spore germination.

4.5   PGPR-Producing Antibiotics and Bacteriocins

One of the most effective mechanisms that a PGPR can employ to prevent phytopathogen proliferation is the synthesis of antibiotics which occurs at the end of the exponential growth phase and usually requires quorum sensing, mediated by *N*-acyl homoserine lactones (AHLs). The production of one or more antibiotics is the

mechanism most commonly associated with the ability of PGPR to act as antagonistic agents against phytopathogens (Glick et al. 2007). Antibiotics encompass a heterogeneous group of organic, low-molecular-weight organic compounds produced by microorganisms that are deleterious to the growth or metabolic activities of other microorganisms (Duffy 2003). Six classes of antibiotic compounds are related to the BC of root diseases: phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides (all of which are diffusible), and hydrogen cyanide (HCN, which is volatile) (Burkhead et al. 1994; Haas and Défago 2005; Berry et al. 2010). Numerous types of antibiotics have been isolated from fungal and bacterial strains, and this diversity includes mechanisms of action that inhibit synthesis of pathogen cell walls, influence membrane structures of cells, and inhibit the formation of initiation complexes on the small subunit of the ribosome (Maksimov et al. 2011). More recently, lipopeptide biosurfactants produced by *Pseudomonas* and *Bacillus* species have been implied in BC due to their potential positive effect on competitive interactions with organisms including bacteria, fungi, nematodes, and plants (de Bruijn et al. 2007; Raaijmakers et al. 2010).

Examples of the use of antibiotics for BC activity are as follows: *Bacillus* sp. produced antibiotics, such as polymyxin, circulation, and colistin, which are effective for Gram-positive/Gram-negative and pathogenic fungi (Maksimov et al. 2011). Strains acting through the production of antibiotics can be isolated by screening on a plate inoculated with the target pathogen. The *B. cereus* UW85 strain, which suppresses *oomycete* pathogens, produces the antibiotics zwittermicin A (aminopolyol) and kanosamine (aminoglycoside), which contributes to the BC of alfalfa damping-off (*Phytophthora medicaginis*) (Stabb et al. 1994; Silo-Suh et al. 1994; He et al. 1994), Fengycin by *B. subtilis* strain F-29-3 used for BC of *Rhizoctonia* disease (Deleu et al. 2008), and iturin A by *B. amyloliquefaciens* strain B94 for BC of *R. solani* (Yu et al. 2002). The antibiotics synthesized by BC pseudomonads include agrocin84, agrocin434, 2,4-diacetyl phloroglucinol (DAPG), herbicolin, oomycin, phenazines, pyoluteorin, and pyrrolnitrin.

The fluorescent pigments producing pseudomonads are known to have a significant role in the suppression of fungal pathogens, apparently via the production of antifungal metabolites such as phenazine-1-carboxylate, DAPG, siderophore, and hydrogen cyanide (HCN) (Haas and Keel 2003; de Souza et al. 2003). Siderophores produced by a number of *Pseudomonas* spp. are attracted for their possible role in the biocontrol of a number of plant pathogens. Hence, siderophores can act as antimicrobial compounds by increasing the competition for available iron in the rhizosphere.

HCN and DAPG are produced by *Pseudomonas* sp. strain LBUM300 for BC of bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) on tomato (Lanteigne et al. 2012), phenazines by *P. aeruginosa* strain PNA1 for BC of root rot (*Pythium myriotylum*) on cocoyam (Tambong and Hofte 2001), pyoluteorin by *P. putida* strain NH-50 for BC of red rot (*Glomerella tucumensis*) on sugarcane (Hassan et al. 2011), 2-hexyl-5-propylresorcinol by *P. fluorescens* strain PCL1606 for BC root rot (*Dematophora necatrix*) on avocado (Cazorla et al. 2006), and phenazines and cyclic lipopeptides by *Pseudomonas* strain CMR12a for BC of root rot (*Rhizoctonia* spp.) on bean (D'aes et al. 2011). Phenazine, produced by

pseudomonads, possesses redox activity and can suppress plant pathogens such as *F. oxysporum* and *G. graminis* (Chin-A-Woeng et al. 2003). In the soils, *P. chlororaphis* PCL1391 strain, isolated from roots of tomato plants, synthesizes phenazine-1-carboxamide, which is able to release soluble iron from insoluble ferric oxides at neutral pH, thus raising the possibility to contribute to iron mobilization (Haas and Défago 2005). Pyrrolnitrin by *P. cepacia* strain B37 was used for BC of dry rot (*F. sambucinum*) on potato (Burkhead et al. 1994), while pyrrolnitrin produced by the *P. fluorescens* BL915 strain is able to prevent the damage of *R. solani* damping-off of cotton plants (Hill et al. 1994). The DAPG produced by pseudomonads, an effective and extensively studied antibiotic, causes membrane damage to *Pythium* spp. and is particularly inhibitory to zoospores of fungal oomycete (de Souza et al. 2003). The BC activity of a number of strains has been shown to be directly related to the ability of the bacterium to produce one of these antibiotics.

Regarding bacteria as BC agents to act as a biological solution, some researchers have highlighted the use of sporulating Gram-positive species such as *Bacillus* and *Paenibacillus* spp., which can confer higher population stability during formulation and storage of inoculant products (Emmert and Handelsman 1999; Kokalis-Burelle et al. 2005). In comparison to the fluorescent pseudomonads, *Bacillus* spp. produced substantially fewer antibiotics. However, an antibiotic that is effective in the laboratory against one strain of a pathogenic agent may not prevent damage to the plant.

Other molecules used in microbial defense systems are bacteriocins that differ from traditional antibiotics; they commonly have a relatively narrow killing spectrum and are only toxic to bacteria closely related to the producing strain. Almost all bacteria may make at least one bacteriocin, and many bacteriocins isolated from Gram-negative bacteria appear to have been created by recombination between existing bacteriocins (Riley and Wertz 2002). The colicins, proteins produced by some strains of *Escherichia coli* that are lethal for related strains, are the most representative bacteriocins produced by Gram-negative bacteria. Other bacteriocins are pyocins from *P. pyogenes* strains, cloacins from *Enterobacter cloacae*, marcescins from *S. Marcescens*, and megacins from *B. megaterium* (Cascales et al. 2007). Bacteriocins from *Bacillus* spp. are increasingly becoming more important due to their sometimes broader spectra of inhibition which may include Gram-negative bacteria, yeasts, or fungi. In addition to Gram-positive species, some of which are known to be pathogenic to humans and/or animals (Abriouel et al. 2011).

Since one of the major ways in which PGPR act as BC agents is through the antifungal phytopathogen activity of the antibiotics that they produce, production of antibiotics by PGPR may be improved by cloning genes that encode antibiotics normally produced by other bacteria. The genetic manipulation increases the amount of antibiotic that the bacterium synthesizes. Hence, it should be possible to extend a broad spectrum of antibiotics against many phytopathogens. The amount of antibiotic produced by a particular bacterium might be obtained by conventional mutagenesis and selection. The more extensive manipulation of antibiotic production will be obtained through the use of recombinant DNA technology.

4.5.1   PGPR Producing Siderophores

Siderophores can be defined as small peptidic molecules containing side chains and functional groups that can provide a high-affinity set of ligands to coordinate ferric ions (Crosa and Walsh 2002). Based on their iron-coordinating functional groups, structural features, and types of ligands, bacterial siderophores have been classified into four main classes (carboxylate, hydroxamates, phenol catecholates, and pyoverdines). Bacterial siderophores are widely recognized and used by different or species-specific microorganisms (Crowley 2006).

Iron is one of the most abundant minerals on the Earth; however, in the soil, it is unavailable for direct assimilation by microorganisms because ferric ion or Fe^{3+} about 10–18 M at pH 7.4 is only sparingly soluble (Neilands et al. 1987). Soil microorganisms secrete iron-binding molecules (siderophore complex) with low molecular mass (400–1000 daltons), which bind Fe^{3+} with a very high affinity (Kd =10–20 to 10–50) and transport it back to the microbial cell where it is taken up by means of a cellular receptor located in the outer cell membrane of the bacterium and then make it available for microbial growth (Boukhalfa and Crumbliss 2002; Andrews et al. 2003). Siderophores have been recognized as an important antagonistic tool for some PGPR by binding most of the Fe3+ that is available in the rhizosphere with high specificity and affinity, making the iron unavailable for pathogens and limiting their growth (Thomashow and Weller 1990; Masalha et al. 2000; Katiyar and Goel 2004; Dimkpa et al. 2009; Gaonkar et al. 2012).

The ability of bacterial siderophores to suppress phytopathogenic organisms is an important trait that could have a significant agronomic impact. Most plants can grow at much lower iron concentrations than microorganisms. Pseudomonads are known for their high affinity to the ferric ion. The potent siderophore, pyoverdin can inhibit the growth of bacteria and fungi that present less potent siderophores in iron-depleted media in vitro (Kloepper et al. 1980). The siderophore of bacteria such as *B. subtilis* CAS15 was linked to BC of Fusarium wilt (*F. oxysporum* Schl. f.sp. *capsici*) on pepper (Yu et al. 2011), and the siderophore of *Pseudomonas* spp. was linked to BC of bacterial wilt (*R. Solanaceae*) on tomato (Jagadeesh et al. 2001).

Fungal phytopathogens also synthesize siderophores but generally have a lower affinity for iron than do siderophores produced by PGPR (Crosa and Walsh 2002), so that PGPR in effect outcompete fungal phytopathogens for available iron. A pseudobactin siderophore produced by *P. putida* B10 strain was able to suppress *F. oxysporum* in soil deficient in iron; this suppression was lost when the soil was replenished with iron, a condition that represses the production of iron chelators by microorganisms (Kloepper et al. 1980). Soilborne fungal pathogens can be suppressed by fluorescent pseudomonads through the release of iron-chelating siderophores (Loper 1988; Paulitz and Loper 1991; Dwivedi and Johri 2003).

4.5.2 PGPR Producing Defense Enzymes

Many plants respond to pathogen attack by synthesizing pathogenesis-related (PR) proteins that can hydrolyze the cell walls of some fungal pathogens (Huang et al. 2005; Xiao et al. 2009). Some PGPR strains have been found to produce enzymes including chitinase, β-1,3-glucanase, protease, and lipase that can lyse fungal cells (Pal and Gardener 2006; Ramyabharathi et al. 2012).

The enzymes chitinase and β-1,3-glucanase produced by *B. subtilis* strain EPCO 16 strongly inhibited *F. oxysporum* f.sp. *lycopersici* on tomato. A strain of *P. stutzeri* produced extracellular chitinase and laminarinase, which could digest and lyse *F. solani* mycelia, thereby preventing the fungus from causing crop loss due to root rot, and were able to reduce the incidence of disease caused by phytopathogenic fungi *R. solani*, *S. rolfsii*, and *P. ultimum* by using a β-1,3-glucanase-producing strain of *P. cepacia*, which was able to damage fungal mycelia. Similarly, chitinase produced by *B. cereus* strain 28–9 was linked to BC of *Botrytis* leaf blight (*Botrytis elliptica*) of lily (Huang et al. 2005).

Three different strains of the BC PGPR *Enterobacter agglomerans* that are antagonistic to fungal pathogens including *R. solani* possess a complex of four separate enzymes that is responsible for the chitinolytic activity of the bacteria. These bacteria significantly decreased the damage to cotton plants following infection with *R. solani*. Moreover, Tn5 mutants of one of these BC strains that were deficient in chitinase activity were unable to protect the plant against damage caused by the fungal pathogen. Since many of the enzymes (including chitinases and β-1,3-glucanases) from BC PGPR that have been found to lyse fungal cells are encoded by a single gene, it should be useful to isolate some of these genes and then transfer them to other PGPR, thereby constructing BC PGPR that produce both antibiotics and fungus-degrading enzymes (Xiao et al. 2009).

4.5.3 PGPR Producing Antifungal Metabolites and Volatile Compounds Involved in Both Plant Growth Promotion and BC

A wide range of low-molecular-weight metabolites with antifungal activity is produced by PGPR (Dowling and O'Gara 1994). Some pseudomonads can synthesize HCN and are able to inhibit some pathogenic fungi. Several different microorganisms including strains of *Cladosporium werneckii*, *P. cepacia* (*B. cepacia*), and *P. solanacearum* are able to hydrolyze fusaric acid compound, the causative agent of the damage to plants infected by *Fusarium*. As a consequence of the ability to hydrolyze fusaric acid, these bacterial strains can prevent the damage that is caused by various species of the fungus *Fusarium* (Van Rij et al. 2005). Cyclolanostan-3-ol, acetate, (3.beta.)-(CAS) cycloartanyl acetate is one of secondary metabolites produced by *B. cereus* 11UJ which had an activity to rice sheath blight and blast (Suryadi et al. 2015). A variety of volatile organic compounds (VOCs) have been shown to be produced by *Bacillus* spp. including 2,3-butanediol, 2-ethyl-hexanol, 2,4-bis (2-methyl

propyl)-phenol, 4-hydroxybenzaldehyde, 2-nonanone, and various volatile blends. VOCs have been implicated in the BC of postharvest decay (*Penicillium crustosum*) on citrus (Arrebola et al. 2010), inhibition of growth and spore germination of *F. oxysporum* f.sp. *cubense* (Yuan et al. 2012), inhibition of mycelial growth of *F. solani* (Li et al. 2015), induction of the systemic resistance to *Erwinia carotovora* subsp. *carotovora* (Ryu et al. 2004), and growth promotion of *Arabidopsis* (Ryu et al. 2003).

4.6   Induced Resistance (ISR and SAR)

The choice of defense strategy may combine the advantages of enhanced disease protection and low costs. Induced resistance can entail costs due to the allocation of resources of defensive products (Bakker et al. 2013). Physiology and metabolic responses are altered after the induction of ISR, leading to the enhanced synthesis of some plant defense chemicals which limit the pathogen. PGPR cause a line of defense against pathogen spread in the plant, such as strengthening the epidermal and cortical cell walls as seen with *B. pumilus* strain SE34 in pea and tomato (Benhamou et al. 1996, 1998) and *P. fluorescens* WCS417r in tomato (Duijff et al. 1997). These biochemical or physiological changes are associated with the accumulation of pathogenesis-related proteins (PR proteins) and defense chemicals including phytoalexins, phenylalanine ammonia lyase (PAL), and chalcone synthase (Ongena et al. 2000; Dao et al. 2011; Mariutto et al. 2011).

Nonpathogenic rhizobacteria have been shown to suppress severity or incidence of disease by inducing a resistance mechanism in the plant termed as induced systemic resistance (ISR) (Van Loon et al. 1998; Jellis 1998; Ramamoorthy et al. 2001). Induced resistance is the state of an enhanced defensive ability developed by plants when appropriately stimulated (Van Loon et al. 1998). *Pseudomonas* and *Bacillus* spp. are the most studied rhizobacteria that trigger ISR (Van Wees et al. 2008). ISR was described in carnation (*Dianthus caryophyllus*) that was systemically protected by the *P. fluorescens* strain WCS417r against *F. oxysporum* f.sp. *dianthi* (Van Peer et al. 1991), while on cucumber (*Cucumis sativus*), rhizobacterial strains protected the leaves against anthracnose caused by *Colletotrichum orbiculare* (Wei et al. 1991).

Rhizobacteria-mediated ISR resembles pathogen-induced systemic acquired resistance (SAR) in that both types of induced resistance render uninfected plant parts more resistant to plant pathogens, including fungal, bacterial, and viral pathogens, as well as nematodes and insect herbivores (Zehnder et al. 1997; Van Loon et al. 1998; Bent 2006; Pozo and Azcón-Aguilar 2007). ISR has also been demonstrated in many plant species, e.g., bean, radish, tobacco, tomato, and *Arabidopsis thaliana* (Durrant and Dong 2004; Ryals et al. 1996; Van Wees et al. 1997; Van Loon et al. 1998).

SAR and ISR protect plants through different signaling pathways. Unlike SAR that is dependent on the salicylic acid (SA) signaling pathway and causes visible symptoms, ISR is dependent on jasmonic acid (JA) and ethylene (ET) signaling pathways and does not cause visible symptoms in the plant (Knoester et al. 1999; Maurhofer et al. 1998; Van der Ent et al. 2009; Van Loon et al. 1998; de Vleesschauwer and Höfte 2009). In line with the development of SAR, SA was accumulated locally

at lower levels. Application of exogenous SA also induces SAR in many plant species (Van Loon et al. 1998). The development of tissue necrosis was used to be considered a common and necessary feature for SAR activation (de Vleesschauwer and Höfte 2009), but SAR can also be triggered without tissue necrosis as demonstrated in *A. thaliana* (Mishina and Zeier 2007). ISR and SAR can act additively in inducing resistance to pathogens. They together provide better protection than each of them alone (Van Wees et al. 2000). The protection mediated by ISR is significantly less than that obtained by SAR, and a degree of dependence on plant genotype is observed in the generation of ISR (Van Loon 2000; Bloemberg and Lugtenberg 2001).

In SAR, the first infection predisposes the plant to resist further attacks. SA activates specific sets of defense-related genes called pathogenesis-related proteins (PRs). The enhanced defensive capacity characteristic of SAR is always associated with the accumulation of PRs (Van Loon 2007). Treatment of tobacco roots with *P. fluorescens* CHA0 triggers the accumulation of SA-inducible PR proteins in the leaves (Maurhofer et al. 1994). Some of these PRs are β-1,3-glucanases and chitinases capable of hydrolyzing fungal cell walls, while other PRs are poorly characterized. SAR-associated PRs suggest an important contribution of these proteins to the increased defensive capacity of induced tissues (Van Loon et al. 1998). The PR-1 gene or protein expression appears to be inducible by SA, and it is usually taken as a molecular marker to indicate that SAR has been induced (Van Loon and Bakker 2006). *Arabidopsis* plants inoculated with *P. syringae* pv. tomato or sprayed with SA developed SAR and accumulated PR-1, PR-2, and PR-5 mRNAs (Pieterse et al. 1996). Plant inoculated with *P. fluorescens* WCS417r or *P. putida* WCS358 developed ISR; however, PR accumulation of PRs was not detected (Van Wees et al. 1997). ISR can be induced in plants that are unable to accumulate SA (NahG mutant plants). In *Arabidopsis*, SA and the activation of PR genes are not part of the ISR pathway (Pieterse et al. 1996).

Transduction of the SA signal requires the regulatory (activator) protein NPR1 (or NIM1) that functions in the terminal part of the signaling pathway of SAR (Van Loon et al. 1998). In non-induced plants, NPR1 is present as a multimer, and during SAR induction, SA triggers the conversion of NPR1 into a monomeric form and translocated to the nucleus (Kinkema et al. 2000; Verhagen et al. 2006). They interact with members of the TGA/OBF subclass of basic leucine zipper (bZIP) transcription factors that are involved in SA-dependent activation of PR genes (Fan and Dong 2002; Zhang et al. 2003). A direct interaction between NPR1 and a specific TGA transcription factor is required for the binding of the complex to elements within the promoter of the PR genes (Després et al. 2000; Fan and Dong 2002). Overexpression of the NPR1 gene leads to enhanced resistance to pathogen attack (Cao et al. 1998; Friedrich et al. 2001). NPR1 regulates defense responses mediated by different signaling pathways that function beyond the expression of PR genes, indicating that SAR and ISR converge at the last part of the signaling pathway (Van Loon et al. 1998). In *Arabidopsis*, the rhizobacterial *P. fluorescens* strain WCS417r demonstrated that WCS417r-mediated ISR functioned independently of SA and depended on NPR1, although requiring components of the JA and ethylene (ET) response pathways (Pieterse et al. 1996, 1998, 2000).

Methyl jasmonate (MeJA)-induced protection is blocked in *jar1*-1, *etr1*-1, and *npr1*-1 plants, whereas the ethylene precursor 1-aminocyclopropane-1-carboxylate

(ACC)-induced protection is affected in *etr1*-1 and *npr1*-1 plants, but not in *jar1*-1 plants. Therefore, WCS417r-mediated ISR follows a signaling pathway in which components from the JA and ethylene response pathways are successively engaged to trigger a defense reaction, regulated by NPR1 (Pieterse et al. 1998).

Infected plants increased their levels of JA and ET as a sign of active defense (De Laat and Van Loon 1982). These signaling molecules coordinate the activation of defense responses and, when applied exogenously, can induce resistance (Pieterse et al. 1998). The dependency of ISR on JA and ethylene is based on enhanced sensitivity to these hormones rather than on an increase in their production (Pieterse et al. 2000). The *Arabidopsis* JA response mutant *jar1* and the ET response mutant *etr1* were tested in the development of ISR. Upon colonization of the roots by *P. fluorescens* WCS417r bacteria, mutants *jar1* and *etr1* were unable to develop ISR against *P. syringae* pv. tomato (Pieterse et al. 1998), illustrating the dependency of ISR signaling on these phytohormones.

One or more bacterial determinant must be recognized by specific plant receptors so that resistance is induced. ISR is induced by metabolites or features of a specific bacterial strain (de Vleesschauwer and Höfte 2009). A bacterial traits operative in triggering ISR have been identified, including cell structures such as flagella (Meziane et al. 2005), cell envelope components like lipopolysaccharides (Leeman et al. 1995), metabolites including SA and siderophores (Van Loon et al. 1998; Höfte and Bakker 2007; Press et al. 2001; Ran et al. 2005), N-alkylated benzylamine (Ongena et al. 2005), surfactin and fengycin lipopeptides (Ongena et al. 2007), VOCs (Ryu et al. 2004), phenolic compounds (Akram et al. 2013), and signal molecules such as N-acyl-L-homoserine lactone (AHL) (Schuhegger et al. 2006; de Vleesschauwer and Höfte 2009). Among these inducers, VOCs may play a putative role in eliciting host defense and growth promotion (Ryu et al. 2004).

Bacterial determinants elicit ISR from the PGPR strain *Ochrobactrum lupine* KUDC1013 and the secreted bacterial compounds phenylacetic acid, 1-hexadecene, and linoleic acid against *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) in tobacco seedlings. The involvement of quorum sensing (QS) in the elicitation of ISR against Pcc and CMV by the PGPR bacteria strain *S. marcescens* 90–166. Fungi such as *T. asperellum* strain SKT-1 can also elicit this defense response-mediated ISR against fungal pathogens and yellow strain of CMV in *Arabidopsis* (Ryu et al. 2003). The ability to develop ISR in response to certain rhizobacteria has been demonstrated in several species of plants (Van Loon et al. 1998) and appears to depend on the specificity of the interaction between rhizobacteria and plants. Failure to elicit ISR in certain hosts may be due to the absence of production of inducing components in the rhizosphere or an inability of the particular plant species to perceive such compounds (Van Loon 2007). For induction of resistance, it is necessary to know specific recognition between the plant and the rhizobacteria. Depending upon plant species, *P. putida* WCS358r and *P. fluorescens* WCS374r act in different ways. For instance, WCS358r elicits ISR in *Arabidopsis* but does not elicit ISR in radish and carnation plants (Van Peer et al. 1991; Van Peer and Schippers 1992; Leeman et al. 1995; Van Wees et al. 1997). WCS374r is responsive to radish, while it is not responsive to *Arabidopsis* plants (Leeman et al. 1995; Van Wees et al. 1997). In *Arabidopsis*,

WCS417r elicits ISR against a variety of plant pathogens such as bacterial leaf pathogens *X. campestris* pv. *armoraciae* and *syringae* pv. *tomato* DC3000 (Pst DC3000), the fungal leaf pathogen *Alternaria brassicicola*, the oomycete leaf pathogen *Hyaloperonospora parasitica*, and the fungal root pathogen *F. oxysporum.*

PGPR induced systemic resistance by activating the signaling pathways in plants, such as SA, JA, or ET signaling pathways (Maurhofer et al. 1998). Different PGPR triggered ISR dependent on different pathways. Several rhizobacteria induced systemic resistances by simultaneously activating SA- and JA−/ET-dependent signaling pathways. The ISR triggered by rhizobacterium *B. cereus* AR156 is both involved in the SA and JA/ET signaling pathways and NPR1 (Niu et al. 2011).

4.7 Current Research Toward the Development of PGPR as BC Agent

4.7.1 PGPR in Management of Biotic Stresses (Phytopathogens)

4.7.1.1 Relationship Between Plant Growth and BC, Broad-Spectrum Defense Activity, Consistent Performance, and Protection of Using PGPR

In plants, biotic stresses, such as pests and diseases, are threatening crop production. These include many species and types of phytopathogens (fungi, bacteria, and viruses) and other organisms. The dependency on inorganic agrochemical pest and disease control in modern farming is responsible for environmental pollution as well as harmful effects on nontarget organisms.

Exploiting naturally occurring PGPR as BC agents to manage the biotic stresses represents one means of addressing the problems associated with agrochemical control. Damages caused by phytopathogens can be reduced by using beneficial soil bacteria (PGPR) via different indirect mechanisms such as the production of antibiotics, metabolites, and defense enzymes, bacterial competition, secretion of iron-chelating siderophores, and induction of systemic resistance (ISR) in plants (Glick 1995; Glick et al. 1999).

Although the beneficial effects of PGPR on plants are usually separated into two categories, growth promotion and BC, there is a close relationship between them (Mariano and Kloepper 2000). PGPR promote the growth of the entire plant, which can result in the plant having increased tolerance to disease and, conversely of plant diseases by PGPR, may indirectly result in the promotion of plant growth (Beneduzi et al. 2012). Hence, individual strains of PGPR have been shown to exhibit both growth promotion and BC through various mechanisms.

In search of efficient PGPR strains, multiple traits related to plant growth and BC activity have been tested together during the screening process, resulting in the identification of PGPR strains that exhibited multiple functions related to crop production (Ahmad et al. 2008; Praveen Kumar et al. 2014; Wahyudi and Astuti 2011). Some PGPR strains have the potential to ISR against multiple plant pathogens (Ramamoorthy et al. 2001). For example, PGPR strains *P. putida* 89B-27 and *S. marcescens* 90-166

both elicited ISR in cucumber against anthracnose caused by *Colletotrichum orbicu-lare* (Wei et al. 1991), Fusarium wilt caused by *F. oxysporum* f.sp. *cucumerinum* (Liu et al. 1995), bacterial angular leaf spot caused by *P. syringae* pv. *Lachrymans* (Liu et al. 1995), cucurbit wilt infected by *E. tracheiphila* (Kloepper et al. 1992), and *Cucumovirus* in cucumber and tomato (Raupach et al. 1996).

4.7.2   Forming Complex Mixtures: Individual PGPR vs. Mixtures of PGPR

The majority of published reports of plant disease BC evaluate single PGPR strains against a single pathogen through one main mechanism (Murphy et al. 2000; Zhang et al. 2010). For example, Huang and his colleagues reported that the anti-biotic-producing bacterium *B. pumilis* strain SQR-N43 directly inhibited damping-off of cucumber, caused by *R. solani*. Antibiotic-producing rhizobacteria exhibiting BC via antibiotic production have been reported with diverse bacteria in various host/pathogen systems, including *B. subtilis* strains NH-100 and NH-160 against red rot of sugarcane, caused by *C. falcatum* (Hassan et al. 2010); *B. subtilis* strains PFMRI, BS-DFS, and PF9 against bacterial wilt of potato caused by *R. sola-nacearum* (Aliye et al. 2008); and *P. fluorescens* strain FP7 against mango anthrac-nose caused by *C. gloeosporioides* (Vivekananthan et al. 2004).

The synergy of different mechanisms produced the same strain BC of diseases, while one prominent BC mechanism was exhibited by a single strain. The extracel-lular enzyme (β-1,3-glucanase) and an antibiotic that was produced by *B. subtilis* NSRS 89-24 played a synergistic role in the control of two fungal pathogens *P. grisea* and *R. solani* on rice (Leelasuphakul et al. 2006).

Single PGPR strains with one main mechanism of action for BC have also been selected based on the production of siderophores and elicitation of induced sys-temic resistance (ISR). The siderophore-producing *B. subtilis* strain CAS 15 com-peted for iron with the soilborne pathogen *F. oxysporum* f.sp. *capsici* and also promoted the growth of pepper (Yu et al. 2011). With ISR, *B. pumilus* strain SE34 induced defense to Fusarium wilt (*F. oxysporum*) (Benhamou et al. 1998) and tomato late blight (*P. infestans*) (Yan et al. 2002).

Despite the positive results, Pal and Gardener (2006) reported that single PGPR strains have not been used on a wide range of plant hosts and have typically exhib-ited inconsistent performance in the field. A single PGPR strain typically does not have BC activity against multiple pathogens. In addition, it is not likely to be active at a high enough level against pathogens under diverse conditions found in the field, including competitive indigenous microorganisms, diverse environmental condi-tions, unpredictable weather, and multiple plant diseases (Elmqvist et al. 2003). The formulation of mixtures of PGPR is one strategy to address multiple modes of action and BC of multiple pathogens (Domenech et al. 2006).

Several studies have shown that compatible mixtures of PGPR strains can pro-vide broad-spectrum activity against different pathogens. Compatible mixtures of PGPR have been shown to induce a higher level of protection than individual PGPR

strains. Mixtures of PGPR exhibited a general trend toward a more consistent and higher magnitude disease suppression than did individual strains of PGPR (Bharathi et al. 2004; Lucas et al. 2009). In addition, some mixtures of PGPR, selected for elicitation of ISR, reduced disease at the same level as a commercially available chemical elicitor (Actigard® Syngenta) (Raupach and Kloepper 1998).

Compatible mixtures of PGPR can give consistent performance. Individual PGPR and mixtures have been tested in Thailand during the rainy season and winter season and showed that mixtures more consistently suppressed both disease severity and disease incidence in both seasons than did individual strains (Jetiyanon et al. 2003). It also demonstrated good efficacy of mixtures for controlling phytophthora blight of pepper under two different field conditions with crop rotation in Korea (Kim et al. 2008).

Ji et al. (2006) used pairwise combinations of three foliar BC agents and two selected PGPR strains against three foliar bacterial pathogens (*P. syringae* pv. *tomato*, *X. campestris* pv. *Vesicatoria*, and *X. vesicatoria*) in tomato. Szczech and Dyśko (2008) mixed three different PGPR strains against two soilborne disease (*F. oxysporum* f.sp. *radices-lycopersici* and *R. solani*) in tomato. A mixture of PGPR was used against different types of pathogens that included a group of fungi (*Macrophomina phaseolina*, *F. solani*, and *R. solani*) and root-knot nematode (*M. javanica*) in tomato (Siddiqui and Shaukat 2002). Raupach and Kloepper (1998) used a two-way or three-way mixture against three different pathogens (*C. orbiculare*, *P. syringae* pv. *lachrymans*, *E. tracheiphila*) in a single host (cucumber). In a study of BC pre-screened in the greenhouse and the field to bacterial wilt of tomato, anthracnose of pepper, damping-off of green kuang futsoi, and cucumber mosaic virus, some PGPR mixtures caused at least a 50% disease suppression of most of these diseases compared to the non-PGPR-treated control treatment (Jetiyanon and Kloepper 2002).

The formulation of strain mixtures is a key approach to increase the efficacy of plant growth promotion and plant disease protection in the field (Choudhary and Johri 2009). Stable formulations using different carriers such as peat and talc have been developed for the delivery of the PGPR stains for field level application. Nakkeeran et al. (2004) used talcum- and peat-based formulations of *P. chlororaphis* and *B. subtilis* for the management of turmeric rhizome rot. Talcum-based strain mixtures were effective against rice ShB and increased plant yield under field conditions greater than did individual strains (Nandakumar et al. 2001).

4.8   Utilization of PGPR on Tea Plant

4.8.1   Induction of Resistance for Management of Blister Blight on Tea Plant Using PGPR

Camellia sinensis (tea) is a tree that is naturally distributed in highland plantation parts of Indonesia. However, most of the tea plant has been damaged due to biotic as well as abiotic factors. In addition, plant growth and survival are affected by the fertility of the soil and by low availability of the nutrients.

The role of tea commodities in the economy in Indonesia is quite strategic; however, the area of tea plantations in Indonesia continues to decline. Tea production is often faced with many factors such as weather and plant pest and disease disturbances. The main diseases in tea plants are blister blight caused by the fungi *Exobasidium vexans* Massee. Blister blight can cause yield losses up to 40–50% and decrease the tea quality lower to 35% (Gulati et al. 1993; Martosupono 1995).

Control of blister blight can be done by various strategies, such as technical culture, resistant clones, and fungicide applications. Control with fungicides (especially copper fungicide) is an effective method to control blister blight. However, the use of copper fungicide continuously can cause a negative consequence such as increasing population of mites (*Brevipalpus phoenicis*) (Oomen 1980; Venkata Ram 1974), cause damage in the soil structure due to the accumulation of copper, and decrease the population of earthworms (Shanmuganathan 1971; Shanmuganathan and Saravanapavan 1978). Therefore, the alternative method in controlling blister blight which is more environmentally friendly is required. An alternative strategy that can be done is BC because this method is appropriate with the concept of sustainable agriculture.

A large number of commonly found microorganisms in the soil (bacteria, fungi, actinomycetes, protozoa, algae, etc.) show the ability to utilize a wide range of beneficial substances (Lynch 1990; Linderman 1992; Glick 1995; Kennedy 1998; Barea et al. 2002). Beneficial root-colonizing rhizosphere bacteria (PGPR) are defined by three intrinsic characteristics: (a) they must be able to colonize the root; (b) they must survive and multiply in microhabitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant promotion/protection activities; and (c) they must promote plant growth (Kloepper et al. 1992; Van Peer and Schippers 1992). The complexity of the soil system is determined by the numerous and diverse interactions among its physical, chemical, and biological components, as modulated by the prevalent environmental conditions. Many microbial interactions, which are regulated by specific molecules/signals, are responsible for the maintenance of plant health and soil quality (Barea et al. 2004).

The potentiality of PGPR in agriculture is steadily increased as it offers an attractive way to replace the use of chemical fertilizers, pesticides, and other supplements (Fatima et al. 2008). A number of different PGPR include *Azotobacter* species, *Azospirillum* species, pseudomonads, *Acetobacter* species, *Burkholderia* species, and *Bacillus* species (Kloepper et al. 1992). The genus *Bacillus* are important PGPR microorganisms that can produce phytohormones, such as auxin and cytokinin, which promote root development (Erturk et al. 2010).

PGPR are important microorganisms that can increase the growth and yield of tea plants; however, there is little information on the beneficial effects of PGPR inoculation on the growth tea seedlings as well as control of blister blight disease caused by *Exobasidium vexans* Massee that can decrease yield loss up to 50% of tea in the field; hence, an effort to reduce blister blight, a major disease in tea plantation, needs to be carried out. Research has been conducted to know the effect of PGPR on tea plant growth that can work optimally as a biological fertilizer and plant resistance inducer to suppress blister blight. The previous study found that bacterial isolates have functioned as biofertilizers and can act as BC agents, namely, *Chryseobacterium* sp. AzII-1, *Acinetobacter* sp., *Alcaligenes* sp. E5, *Bacillus* E65,
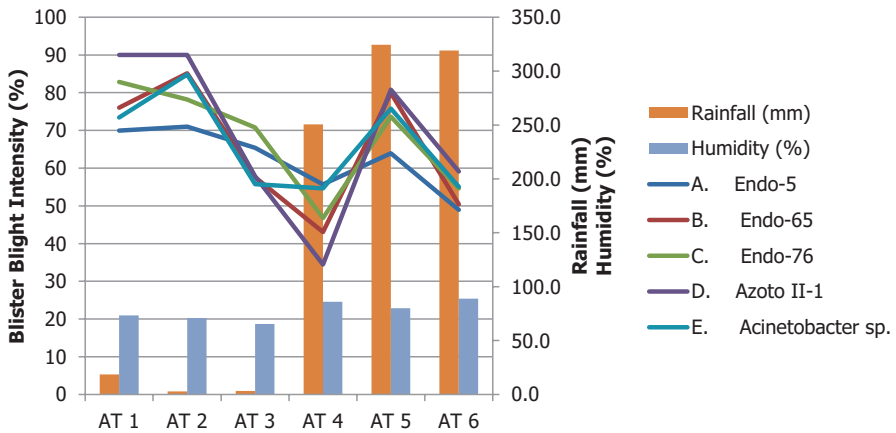
Fig. 4.1 Rainfall, humidity, and intensity of blister blight during the experiment

and *Burkholderia* E76. Molecular characterization results also indicate that the bacterial isolates have survival capabilities in both biotic and abiotic stress conditions and did not cause necrosis in plants, and the detection of the presence of IAA-coded gene genes was also found (148 bp) (Rachmiati 2015).

The experiment conducted at Gambung Experimental Garden, Research Institute for Tea and Cinchona, West Java, Indonesia, using TRI 2024 clone was done to determine the effect of microbial application to induce plant health against blister blight. The preliminary observations showed that, at the beginning of the trial, the condition of blister blight was homogeneous. At the initial condition (at the third preliminary observation) before the treatment application, the average of disease intensity was ±72.67%. In general, during the experiment, the pattern of disease intensity fluctuated. Figure 4.1 showed that all blister blight intensity decrease in all treatments after the first application. The disease intensity consistently decreased from the first (AT 1) observation to the fourth (AT 4) observation. However, the intensity of the disease increased after the fifth (AT 5) observation. The intensity of blister blight remained high until the last observation, with an average of disease intensity 53.63%. This condition may be influenced by rainfall or leaf wet conditions (high humidity and misty). The amount of rainfall and humidity at the end of observation was 319 mm and 89%.

The environmental conditions support the development of the disease. The rainfall and humidity conditions during the experimental period affect the intensity of blister blight disease. The fluctuations of the intensity of blister blight disease in line with the amount of rainfall and the average of humidity on every observation. Therefore, the intensity of blister blight disease is still high until the final observation.

It showed that the microbial treatment on cumulative of tea fresh shoot did not significantly change (Table 4.1). However, the cumulative tea fresh shoot on the *Acinetobacter* sp. was 17.26% higher when compared with other treatments. The decrease in the intensity of blister blight was not accompanied by increased yield of fresh shoots. The intensity of blister blight was >50% until the end of observations. The yield loss caused by blister blight does not relate quantitatively to disease control.

Table 4.1 Results of cumulative of tea fresh shoot on various microbial treatments

Treatment	Cumulative of fresh shoot (kg/plot)[a]	Yield increase (%)
A. *Alcaligenes* sp. E5	2.014	−1.09
B. *Bacillus* E65	1.907	−6.33
C. *Burkholderia* E76	2.145	5.35
D. *Chryseobacterium* sp. AzII-1	2.132	4.68
E. *Acinetobacter* sp.	2.388	17.26
Significance	NS	

[a]Cumulative from six times of application

Table 4.2 Average of bacterial population (CFU/ml)

Combination	Average of *Azotobacter* sp. population (CFU/ml)	Average of endophytic bacteria population (CFU/ml)	Average of total bacteria population (CFU/ml)
A. *Chryseobacterium* sp. AzII-1 + *Acinetobacter* sp.	2.78×10^8	2.57×10^8	5.35×10^8
B. *Chryseobacterium* sp. AzII-1 + *Alcaligenes* sp. E5	2.09×10^8	1.31×10^8	3.40×10^8
C. *Chryseobacterium* sp. AzII-1 + *Burkholderia* E76	2.51×10^8	2.48×10^8	5.00×10^8
D. *Chryseobacterium* sp. AzII-1 + *Bacillus* E65	2.19×10^8	2.55×10^8	4.74×10^8
Significance	NS	NS	NS

NS nonsignificant

The TRI 2024 clones in this study are susceptible to blister blight. In general, the application of the inducer agent causes the plant to become rapidly sensitive in response to pathogen infection. Moreover, endophytic bacteria have several benefits including the N_2 air-inhibitor, producing phytohormones such as indole-3 acid (IAA), cytokinin, and stimulate the growth (Setiawati et al. 2009). The test results are used as the basis for determining the combination of active ingredients for biofertilizer.

The four formulas are not significantly different in populations of *Azotobacter* sp., endophytic bacteria, as well as total bacteria (Table 4.2). This means that the four formulations were a synergist. According to the Indonesian Ministry of Agriculture Regulation No. 70 of 2011 on Organic Fertilizer, Biological Fertilizer, and Soil Enhancer, the minimum required population of compound biochemical fertilizer was 10^7 CFU/g.

The combination of *Chryseobacterium* sp. AzII-1 + *Alcaligenes* sp. E5 was tested under tea plant nursery. The intensity of blister blight during the trial was very low. This might be due to high temperatures during the experiment (dry season); however, the blister blight intensity in treatment D (*Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25%) was significantly different compared with the other treatments, with disease intensity at final observation of 1.27% (Table 4.3). The results of the biochemical analysis showed that *Chryseobacterium* sp. AzII-1 and

Table 4.3 The intensity of blister blight in various treatment combinations of bacteria

Treatment	The intensity of the disease (%)*
A. Control (without bacteria)	1.84%[ab]
B. *Chryseobacterium* sp. AzII-1 25% + *Alcaligenes* sp. E5 75%	1.84%[ab]
C. *Chryseobacterium* sp. AzII-1 50% + *Alcaligenes* sp. E5 50%	2.09%[b]
D. *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25%	1.27%[a]
E. *Chryseobacterium* sp. AzII-1 25% + *Burkholderia* E76 75%	1.85%[ab]
F. *Chryseobacterium* sp. AzII-1 50% + *Burkholderia* E76 50%	2.08%[b]
G. *Chryseobacterium* sp. AzII-1 75% + *Burkholderia* E76 25%	2.19%[b]

∗Mean in the column followed by the same letter is not significantly different according to Duncan's multiple range test at 5%
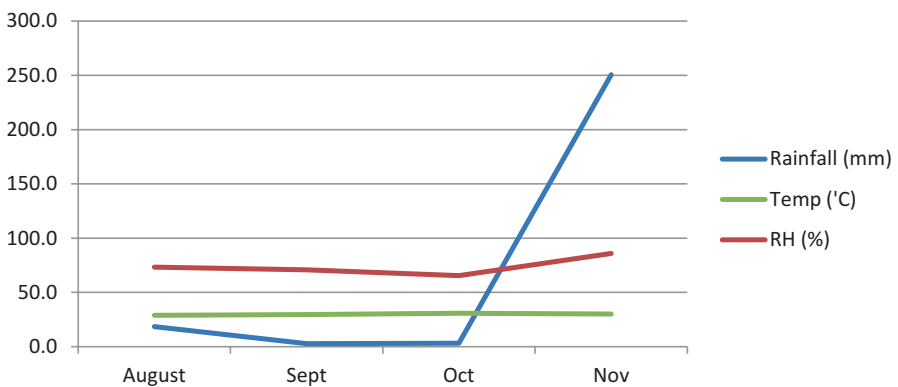
Fig. 4.2 Climate condition during experiment

Alcaligenes sp. E5 had a positive value of chitinase. The disease intensity can be suppressed by the activity of chitinase produced by *Chryseobacterium* sp. AzII-1+ *Alcaligenes* sp. E5. This indicates that the isolates *Chryseobacterium* sp. AzII-1 and *Alcaligenes* sp. E5 are potential as a BC agent against pathogenic fungi.

The climate or weather changes will affect pathogens before infecting plants (pre-penetration). Pathogens are highly sensitive to environmental changes, and their development is determined by the optimum climatic or weather conditions. Environmental conditions during the trial do not support the development of blister blight. The average temperature and humidity approached to 30 °C and 80%, respectively. Although the rainfall and humidity are quite high at the final experiment, it did not affect blister blight development until the end of the trial period (Fig. 4.2). The relationships between rainfall, temperature, and humidity to the

intensity of blister blight show a strong linear regression pattern, which strongly supports that blister blight intensity decreases with decreasing intensity of rainfall, rising temperatures, and low humidity (Rezamela et al. 2016). The formation and spread of basidiospores require higher relative humidity above 80%. Meanwhile, for spores germination required moisture higher than 90% (Astuti 2013).

The combination of *Chryseobacterium* sp. AzII-1 + *Alcaligenes* sp. E5 also affected the tea plant growth. The parameter of plant height is one of an important factors in determining which tea planting material is ready for planting. Stem diameter measurements were performed at 4-month-old plants after planting or 1 month after bacterial applications. The parameters of diameter of stem provide an overview of the growth and development of tea planting material. Moreover, the leaf is one of the components of growth which is directly related to the process of photosynthesis (Table 4.4).

The interesting result showed that all combinations of treatments can affect plant growth. However, in the combined treatment of *Chryseobacterium* sp. AzII-1 + *Alcaligenes* sp. E5, the average of plant growth was higher than that of other treatments. In addition, the higher percentage of *Alcaligenes* sp. E5 can increase plant growth. The average plant height, stem diameter, number of leaves, root length, and root volume were also higher. However, the higher the percentage of *Chryseobacterium* sp. AzII-1, the lower the intensity of blister blight disease.

Using *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25% treatment, the intensity of blister blight disease was the lowest when compared to other treatments, but it does not affect plant growth significantly. The plant height, stem

Table 4.4 The effect plant growth tea planting at the age of 6 weeks after application

Treatment	Plant height (cm)*	Stem diameter (cm)*	Number of leaves*	Root height (cm)*	Root volume (cc)*
A. Control (without bacteria)	12.6[a]	3.2[abc]	7.9[b]	16.40[a]	1.87[ab]
B. *Chryseobacterium* sp. AzII-1 25% + *Alcaligenes* sp. E5 75%	16.93[b]	3.46[c]	9.9[c]	20.08[a]	2.50[b]
C. *Chryseobacterium* sp. AzII-1 50% + *Alcaligenes* sp. E5 50%	14.42[ab]	3.33[abc]	8.4[b]	19.92[a]	2.75[b]
D. *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25%	15.32[b]	3.38[bc]	8.05[b]	18.25[a]	2.37[ab]
E. *Chryseobacterium* sp. AzII-1 25% + *Burkholderia* E76 75%	12.35[a]	3.17[abc]	7.05[ab]	19.62[a]	2.25[ab]
F. *Chryseobacterium* sp. AzII-1 50% + *Burkholderia* E76 50%	12.59[a]	3.13[ab]	6.4[a]	19.77[a]	1.50[a]
G. *Chryseobacterium* sp. AzII-1 75% + *Burkholderia* E76 25%	12.66[a]	3.06[a]	7.75[ab]	19.90[a]	2.62[b]

*The figures in the column followed by the same letter are not significantly different according to Duncan's multiple range test at 5%

diameter, leaf number, root length, and root volume in *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25% treatment were 15.32 cm, 3.38 cm, 8.05, 18.25 cm, and 2.37 cc, respectively.

The gene detection of the presence of IAA growth hormone on *Chryseobacterium* sp. AzII-1 and *Alcaligenes* sp. E5 bacteria was found about 148 bp in size. That means that they have potential as a biofertilizer agent with the ability to produce auxin substance growth boosters. The number of leaves is expected to increase the ability of leaves to photosynthesize. If the rate of photosynthesis increases, the growth rate will be the maximum. The rate of root and shoot growth is influenced by internal factors, such as the supply of photosynthesis from leaves, and environmental factors, such as temperature and soil water content. Endophytic bacteria that produce PGPR can benefit plants through improved root function and accelerate plant growth.

Combining soil bacteria and endophytic bacteria as the active ingredient of biofertilizer can increase the effectiveness of biofertilizer. With a combination of both, biofertilizer can work optimally both as a biological fertilizer and plant resistance inducer. Therefore, a test of synergism was done in the laboratory first before applying in the field. PGPR may affect plant growth in a variety of ways (Glick 1995; Glick et al. 1999). The application of PGPR inoculation is an effective method to improve the growth and nutrient uptake of tea seedlings due to the combined actions of nutrient enhancement systems and root development.

The tea plant rhizosphere bacterial communities which are infected with *Exobassidium vexans* Massee and treated by *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25% have also been monitored. In the rhizobacterial communities of control treatment samples without *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25% through culturing method, the following bacteria were found: *Bacillus* sp. (51.91%), *Acidobacteria bacterium* (39.42%), and *Actinobacteria* sp. (8.66%). In the control treatment through metagenome analysis, the following bacteria were found: *Gemmatimonas aurantiaca* (5.80%), *Bacillus* sp. (42.55%), *Acidobacteria bacterium* (23.45%), and *Actinobacteria* sp. (28.20%). In the communities treatment samples of *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25% treatment, the following bacteria were found: *Gemmatimonas aurantiaca* (3.58%), *Bacillus* sp. (30.76%), *Pseudomonas* sp. (5.55%), *Acidobacteria bacterium* (13.94%), and *Actinobacteria* sp. (46.16%). In the communities of rhizobacteria treatment samples with *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25% treated by metagenome, the following bacteria were found: *Bacillus* sp. (10.66%), *Acidobacteria bacterium* (4.22%), *Actinobacteria* sp. (5.48%), *uncultured bacterium* (1.49%), *Alcaligenes* sp. (36.95%), and *Chryseobacterium* sp. (46.82%). The existence of *Alcaligenes* sp. and *Chryseobacterium* sp. shows the consistency of *Chryseobacterium* sp. AzII-1 75% + *Alcaligenes* sp. E5 25% application in tea rhizosphere plant.

4.8.2 PGPR in BC Management of Diseases on Rice

More than 70 diseases caused by fungi, bacteria, viruses, or nematodes have been recorded on rice. The diseases are the most significant limiting factors that affect rice production, causing estimated annual yield losses of 5% (Manandhar et al. 1998). In Indonesia in each annual planting season, pests and diseases were causing yield losses of 212,984 ton of rice.

Among rice diseases, rice blast (*P. oryzae*) and bacterial blight (BB) of rice caused by *X. oryzae* pv. *oryzae* (*Xoo*) are considered as the major problems for the rice cultivation in both lowland and upland rice in most of rice-growing countries and becoming a serious constraint to rice productivity (Song et al. 2001). The infected area by BB is second largest after rice tungro disease. Yield loss was estimated at about 20% to 50% in the severely infected field and up to 10–20% when the disease infected rice at maximum tillering stage.

The use of pesticides is costly as well as environmentally undesirable. Current control strategies of BB disease mostly make use of resistant cultivars, which is an economical and effective method of control. Due to the breakdown of resistance against high pathogenic variability of the pathogen population, there is a need to develop more strategies providing durable resistance over a broad geographic area to improve the life span of resistant cultivars (Manandhar et al. 1998).

Currently, considerable attention has been given on the use of BC agents using PGPR to suppress plant diseases. Since BC is a key component of integrated disease management, it is important to search for PGPR active against diseases and evaluate this PGPR for BC application under field conditions. The PGPR microbes suppressed the pathogen by various mechanisms such as the production of chitinase and β-1,3-glucanase (Zhang and Yuen 2000) and antibiotic (Nalisha et al. 2006) and by induction of systemic resistance (Saikia et al. 2006). In addition to the more common antibiosis mechanisms, there are a number of other ways in which PGPR can inhibit phytopathogens. For example, competition for nutrients and suitable niches on the root surface may protect plants from phytopathogens in different plant species (Compant et al. 2005)

Many PGPR with a wide range of root-colonizing bacteria can enhance plant growth by increasing seed emergence, plant weight, and crop yields (Kloepper and Beauchamp 1992) and influence plant health by suppressing the growth of plant pathogens (Compant et al. 2005). Most of PGPR bacteria produce phytohormones (auxins, cytokinins, and ethylene) in the rhizosphere that regulate and promote root growth. When soils are alternately flooded and drained, certain bacteria are able to double the size of plant root systems by their activity to contribute on plant growth, increasing biological N fixation and P solubilization (Glick 1995).

Studies on BB control using PGPR had been reported and reviewed in Indonesia (Agustiansyah et al. 2010). The combination of matrix conditioning plus a BC agent (isolate A6) reduced *Xoo* population in rice plants and improved viability and vigor of rice seeds in the glasshouse. The seed treatment and foliar spray application at 2-week interval on rice using *B. subtilis* B12 with 2% concentration showed good result in controlling BB and promoted plant growth at the greenhouse experiment. The application also showed a better effect on suppressing the BB disease as well as increasing

yield in the field experiment. Applications of *B. subtilis* B12 spore formulation reduced BB disease by 21% and increased yield up to 50% (Wartono et al. 2014).

Gram-negative bacteria such as *Lysobacter* spp. were reported inhibiting a fungal pathogen *Bipolaris sorokiniana* in the field (Kilic-Ekici and Yuen 2004). Bacterial isolate *Pseudomonas veronii* PBR 3b had potential ability to hydrolyze β-glucan. *P. aeruginosa* C32a also produced the largest clear zone with the glucanolytic index of 2.27, with temperature and pH optimum for glucanase activity of *P. aeruginosa* C32a at 40 °C and pH 6, respectively. The antagonistic test of *P. aeruginosa* C32a against *P. oryzae* and *R. solani* showed inhibition zones of 59.11% and 37.33%, respectively. This pseudomonad isolate could be promising for BC with broad-spectrum phytopathogens (Suryadi et al. 2014).

Strains of *P. aeruginosa* could induce rice resistance against sheath blight (ShB) by producing different antifungal activities (salicylic acid and peroxidase content) (Saikia et al. 2006). *B. cepacia* isolate E76 treatment was effective in suppressing the growth of *R. solani* with relative inhibitory at 24 and 48 hours after incubation ranging from 31.3% to 60.2% and 28.9 to 47.8%, respectively. Rice germination and growth of treated rice seeds were better than that of control treatment. Suspension formulation of *B. capacitate* 3% concentration was suggested to be used as the recommended concentration for further testing (Wartono et al. 2012). The bacterial culture filtrate *Burkholderia* sp. E76 isolate could inhibit radial growth of fungal colonies with the *R. solani* inhibition ranging from 32.9% to 99.4%. Based on chitinase assay, it was indicated that Gram-negative bacteria of *Burkholderia* sp. E76 isolate produced the highest chitinolytic index (Suryadi et al. 2013a). Four bacterial isolates (C 32a, C 32b, I. 21, and I. 5) could inhibit *R. solani* growth. *B. firmus* E 65 and *P. aeruginosa* C 32b have an excellent potential to be used as BC agents of *R. solani* on rice at the greenhouses when treated as pretreatment spraying application (Suryadi et al. 2011).

On rice cultivation with respect to BC of rice blast disease, there are complex interactions between rhizobacteria and rice plants depending upon both rice cultivar and soil type. A study in Pakistan was reported that 16 bacterial strains isolated from the roots and rhizosphere of rice plants growing in saline and nonsaline soils were tested for their ability to promote plant growth and reduce the incidence of rice blast (Naureen et al. 2009). Several strains inhibited the growth of the *Magnaporthe grisea*, the causal agent of rice blast at in vitro dual culture assay. However, when applied to the soil, many of the isolated rhizobacterial strains increased seedling growth and/or suppressed rice blast disease in greenhouse-grown plants of the cv. Super Basmati and cv. Azucena, but each cultivar responded to different subsets of the bacteria. Blast resistance was increased and correlated with the production rhizobacterial siderophores on cv. Super Basmati. Direct antagonism was correlated with disease resistance in cv. Super Basmati, but not in cv. Azucena, and direct antagonism as a cause for the reduced disease incidence is also unlikely since no epiphytic colonization of leaves was detected. In addition, there were also differences in the ability of some strains to protect plants against blast depending on soil type. Rhizosphere colonization by the bacteria in plants grown in sterile sand was correlated with disease resistance in Super Basmati, but not in cv. Azucena (Naureen et al. 2009).

In Indonesia, 14 endophytic fungi isolated from rhizoplane showed antibiosis activity against *P. Oryzae* under in vitro inhibition test (Sucipto et al. 2015). Several bacteria such as *B. cereus* II.14, *B. firmus* E65, and *P. aeruginosa* C32b produced chitinase and IAA growth hormone, while *B. firmus* E 65 isolate was very effective in suppressing *P. oryzae* (18.15%) blast disease (Suryadi et al. 2011). The formula A2 (*B. firmus* E65) and A6 consortium (*B. firmus* E65, *B. cereus* II.14, and *P. aeruginosa* C32b) significantly reduced the mycelial growth of *P. oryzae* with the percentage inhibition of 73–85% and 66–83%, respectively (Suryadi et al. 2013b).

The ethyl acetate extracts of the *B. cereus* 11UJ showed a better antifungal activity to *P. Oryzae* than those of *R. solani*. The inhibitory effect of the filtrate proved the potency of the isolates to produce antifungal activity. Analysis of pyrolysis gas chromatography mass spectrometry showed that *B. cereus* 11UJ produces three major compounds, i.e., 9,19-cyclolanostan-3-ol, acetate, (3.beta.)- (CAS) cycloartanyl acetate (13.14%), 4-(2′,2′-dimethyl-6′-methylidene-1′-cyclohexyliden)-3-methyl-2-butanone (9.72%), and stigmas-5-en-3-ol oleate (9.09%) which suggested to play an important role in the suppression of rice fungal pathogens (Suryadi et al. 2015).

4.8.3   Development of PGPR Bioformulation to Control Rice Disease Under Organic Cultivation

PGPR could change in microbial population associated with system of rice intensification (SRI) practices. Rhizosphere of SRI soils provides a conducive environment for the proliferation of antagonistic bacteria that promote plant growth (Gani et al. 2002). In line with organic SRI practices, BC using local microorganisms can be applied to contribute its effectiveness in the field. In the previous study, the applications of an individual antagonistic bacterium such as E 65, C 32a, C32b, and E 31 isolates suppressed BB lesion length in the screen house test. Research on BC to BB using microbial agents such as *Bacillus* spp., *Serratia* spp., *P. aeruginosa*, and *Corynebacterium* spp. had been done extensively in the field (Suryadi et al. 2013a). The efficacy of consortium bacteria containing a mixture of bacterial antagonist for controlling major rice diseases was tested under SRI practices. The experiment consists of three consortium bacteria, viz., C1 (*Bacillus* sp. E64 + *B. firmus* E65 + *Burkholderia* sp. E76 + *B. cereus* C29d + *B. licheniformis* CPKPP35 + *Bacillus* sp. H + *Bacillus* sp. IR), C2 (*Bacillus* sp. E64 + *B. firmus* E65 + *Burkholderia* sp. E76 + *B. cereus* C29d + *B. licheniformis* CPKPP35 + *Azospirillum* sp. Aj.5252), and C3 (*Bacillus* sp. H + *Bacillus* sp. IR). The candidate's C1 could reduce the BB and red stripe diseases severity when compared with control treatment (untreated plots), with the efficacy control less than chemical control, although not effective against sheath blight disease. The yield increase obtained by C2 and C3 consortium applications ranges from 8.7% to 12.2% (Suryadi et al. 2013a).

The main factors responsible for the yield enhancement in SRI management were longer panicles with more grains, better grain filling, and a significant increase in grain weight (Thakur et al. 2010). The present study indicates that use of formulation bacteria tends to improve rice yield up to 8% compared with that of the untreated plot

(without formulations). This result may have been due to the indirect effect of antagonism as well as competitions with *Xoo* pathogens for essential nutrients. Further study on the use of bacterial consortium to BB disease is needed by developing suitable delivery technology specific for certain microorganism use as BC agents.

With regard to pathogen reduction, this may probably take place in anaerobic conditions which indicate that minimum amount of oxygen present in the facultative anaerobic condition (static condition) was still needed for the consortium to maintain their basic cellular activity. All isolates incubated in the mixed culture could reduce disease severity, suggesting some degree of synergism; nevertheless, the percentage of BB reduction by consortium formulation was slightly higher than those of cv. Inpari 10-(SKM kaolin), cv. Inpari 13-(E76-bentonite), and cv. Sintanur-(A2-bentonite), but lower compared with cv. Code-(A2-bentonite) treatments.

Inconsistent performances of the microbes in the field, however, had limited their commercial uses; hence, combining several modes of actions against the pathogen could improve their effectiveness. Currently, the uses of bioformulations of the bacterial mixture are gaining great interests in the BC method, and the products are used as a supplement or as an alternative to the chemical control (Gnanamanickam 2009).

We are working toward commercial development of PGPR as a method for both plant growth promotion and BC. Many greenhouse studies and field experiments have been conducted to show the efficacy of PGPR in disease management, but there are still relatively few commercial applications of PGPR for this purpose. Bentonite formulation showed a good effect in suppressing bacterial blight lesion length in the greenhouse test. Talc-A5 formulation (*B. firmus* E 65 + *P. aeruginosa* C32b) was effective against sheath blight and BB but showed the lower effect on neck blast disease in the field (Suryadi et al. 2013b).

The establishment of a mix culture containing at least four distinct bacterial species are encouraging to be applied for the suppession of rice blast pathogen (*P. oryzae*) (Suryadi et al. 2013b). The higher capabilities of consortium A8 and A6 to inhibit BB pathogens within a period of observation indicated that mixture culture isolates might be capable of reducing BB inoculums. One bacterial isolate may be able to cause an inhibition of one pathogen, which consequently renders it more accessible to another organism that otherwise is unable to reduce BB pathogen.

The advantages of single or mixed cultures are apparent, and further exploitation of selected bacterial consortium will be beneficial to suppress BB in the field. BC efficacy among different rice cultivars showed BB disease reduction ranging from 10.5% to 29.4%. The consortia A6 (*B. cereus* II.14 + *B. firmus* E65+ *P. aeruginosa* C32b) and A8 (*B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b + *S. marcescens* E31) with bentonite carrier reduced BB infections up to 25%. The performance of consortium A6-bentonite formulation also gave a better effect than the individual isolate, such as that with *Burkholderia* sp. E76 or *S. marcescens* SKM. The use of consortium bacterial formulation increased rice yields up to 8% than that of the untreated plot.

In controlling rice diseases, it is important to develop synthetic chemicals and minimize the dependence on pesticides. The use of stable bacterial formulations may have been practical in terms of efficacy as well as survival rates. The bacterial isolates were used to prepare basic ingredients for kaolin-based bioformulation

Table 4.5 Effect of bioformulation of PGPR to BB disease and rice grain weight of cv. Inpari 13 at 14 DAI in the GH test

PGPR treatment	Mean of BB lesion length∗ (cm)	BB disease reduction compared to chemical (%)	Grain dry weight (g/pot)∗
SKM + kaolin	8.6[ab]	(11)	5.6[a]
E65+ kaolin	8.4[ab]	(12)	5.3[a]
E76+ kaolin	6.8[b]	9.3	11.2[b]
Without bioformulation application (control)	9.20[a]	(17)	5.0[a]
Copper sulfate (CuSO$_4$) 2%	7.5[b]	–	5.2[a]

∗Means followed by the same letter are not significantly different according to DMRT, $P = 0.05$

grown in NA medium. The bacteria grew well after 24–48-h incubation at room temperature as shown by suitable conditions of the bacterial growth curve for each isolate. A stable bioformulation was very important as a basis for the development of environmentally friendly biocontrol agents to replace the use of synthetic chemicals. All bacterial isolates previously showed being effective in suppressing the growth of fungal pathogens *R. solani* and *P. oryzae* (Suryadi et al. 2011). The ability of isolates varied in suppressing BB lesion length at 14 DAI. A kaolin-based formulation containing *Burkholderia* sp. E76 isolate showed the highest BB disease reduction (9.3%) than that of chemical compounds (CuSO$_4$) (Table 4.5).

Kaolin-based formulations showed good effect in suppressing BB lesions on rice. The addition of bentonite and CMC to bioformulations was fairly stable. The PGPR based on kaolin formulation showed similar effects with bentonite or talcum powder, besides it was easy and cheap, it can be further developed as an alternative carrier. Aside from being able to suppress BB disease, E76 kaolin-based formulation showed the good effect on grain dry weight/pot (Table 4.6). E65 and SKM in kaolin-based formulation had no effect on grain dry weight. Nandakumar et al. (2001) reported that field application of BC agents using *P. fluorescens* isolate could increase rice yields.

The efficacy of bioformulation in the field test showed varied results. BB typical symptoms occurred at the generative stage as shown by leaf blight disease symptoms on rice leaves. The treatment formulation had a lower BB intensity than that of the control treatment (untreated plot). The BB intensity on farmer's rice plot sprayed by bioformulations ranged from 9.7% to 19.4%. In general kaolin-based formulations could reduce the intensity of BB more than 50%. Kaolin-based formulation treated on cv. Inpari 20, cv. Inpari 14, cv. Mekongga, and cv. Sintanur showed BB intensity ranging from 3.3% to 5.55% with the percentage inhibition ranging from 85.2% to 100% compared to controls without the application on cv. Ciherang. It was indicated that on rice treated with the bacterial formulation, the BB intensity has decreased about 84.7% compared to the control treatment without an application that might indicate higher efficacy. Application of bioformulation had no significant effect on

Table 4.6 Effect of mix application of PGPR kaolin bioformulations to the intensity of BB on rice cv. Sintanur

Treatment	Mean of BB intensity (%)	Inhibition over control (%)[a]
Plot farmer 1 (cv. Sintanur + BFM)	19.4	70.9
Plot farmer 2 (cv. Sintanur + BFM)	12.5	81.25
Plot farmer 3 (cv. Sintanur + BFM)	19.4	70.9
Plot farmer 4 (cv. Sintanur + BFM)	9.7	85.45
Plot farmer 5 (cv. Sintanur + BFM)	16.36	75.47
cv. Sintanur without BFM (control)	66.7	–

[a]Inhibition = control − treatment/control × 100%. Sample plots were determined diagonally. Bioformulation of BFM mix containing PGPR SKM, E76 and E65 isolates in kaolin-based ratio (1:1:1) ($^w/_w$) BFM/bioformulation mixture

Table 4.7 Effect of PGPR formulation on plant height, number of tillers, number of panicles, and grain yield

PGPR bioformulation	Number of cells (CFU/ml)				Viability loss (%)[a]
	0 mo	1 mo	2 mos	3 mos	
Kaolin E 65	1.4×10^9	8.3×10^8	4.2×10^8	2.1×10^8	9.07
Kaolin E 76	4.2×10^9	4.2×10^8	3×10^8	1×10^8	16.84
Kaolin SKM	6.4×10^9	4.2×10^8	4×10^8	2.2×10^8	14.98
Mean					13.63 ± 4.05

[a]Viability loss (VL) was calculated using the formula VL = IV−FV/IV × 100%, where IV = initial viability, FV = final viability

plant height, number of tillers, and number of panicles. The highest mean of grain yield (1×1 m^2) was shown on cv. Sintanur with an average of 413.67 g (Table 4.7).

Viability observations to bioformulation were done by counting the number of live cells based on total plate count method. Formulation seems slightly decreased, despite the decrease in cell viability which was not too drastic. The viability of bacterial isolates at the beginning approximately reached an average population of 1.4×10^9 CFU/mL. During the process of storage at room temperature, a visible cell number of bioformulation tended to decrease with an average of 5.5×10^8 CFU/mL at the first month of storage. At the second month of storage, 3.7×10^8 CFU/mL was reached, while at the final observation of 3 months of storage, the population reached 1.76×10^8 CFU/mL (Table 4.8). The mean average of viability loss was approximately 13.63% (Suryadi et al. 2013b).

A range of different molecules has been identified as elicitors of ISR in different systems, including conserved effectors such as flagellar peptides, lipopolysaccharides, antibiotics, cyclic lipopeptides, and siderophores (Compant et al. 2005; Van Wees et al. 2008). Recently, the siderophore pseudobactin was found to be an

Table 4.8 Bacterial cell viability test of formulations after 1-, 2-, and 3-month storage

Treatment	Plant height (cm)*	No. of tiller*	No. of panicles*	Grain yield (g)**
cv. Sintanur + BFM	107.3	24	23.4	413.67 [a]
cv. Inpari 14 + BFM	98.3	18.4	18.2	333.33 [b]
cv. Mekongga + BFM	99.2	20.3	20.3	336.67 [b]
cv. Inpari 15 + BFM	97.1	17.2	17.3	356.67 [b]
cv. Ciherang (untreated)	98	20	23.7	366.67 [b]

Noted: *Not significant; **Means followed by the same letter are not significantly different according to DMRT $P = 0.05$. Grain yield was calculated from rice plot of 1×1 m^2 with a spacing of 30×30 cm. BFM = bioformulation mixture (E65, SKM, E6)

important determinant of ISR against blast disease in rice. They also observed that there was not necessarily any relationship between the ability of a bacterium to inhibit a fungal pathogen when the bacterium was grown in vitro on media that favored the production of either antibiotics or siderophores and the BC activity of the bacterium in vivo (Stephens et al. 1993).

Application of some PGPR strains to seeds or seedlings has also been found to lead to a state of ISR in the treated plant (van Loon et al. 1998; Kloepper et al. 2004). The seed that was treated using seed PGPR applications containing species of *P. fluorescens*, *P. putida*, *B. pumilus*, and *S. marcescens* could affect root system colonization and protect plants against foliar diseases (Liu et al. 1995; Raupach et al. 1996; Kloepper et al. 2004; Pieterse et al. 2000). ISR occurs when the plant's defense mechanisms are stimulated and primed to resist infection by pathogens (Van Loon 2000).

The phenyl propanoid component, salicylic acid (SA), appears to be a critical plant messenger of pathogen exposure and disease resistance, whereas jasmonic acid (JA), a lipoxygenase pathway product, is a potent regulator that mediates plant responses to mechanical damage and pathogenesis (Fan and Dong 2002). The role of microbial volatile organic compounds (VOCs) in regulating plant growth and development has been reported. The bacterial volatile components can serve as agents for triggering growth promotion in *Arabidopsis* (Ryu et al. 2003). Several genera of PGPR strains were assessed for eliciting ISR by volatiles under in vitro conditions. The volatiles produced by selected PGPR strains *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a were characterized, and the effects of volatiles produced by PGPR strains for eliciting ISR at different exposure times and the response of the volatiles to different mutant lines of *Arabidopsis* have been evaluated. The PGPR strains were shown previously to elicit ISR on several crops against fungal, bacterial, and viral pathogens under greenhouse and field conditions. ISR elicited by volatile chemicals was released from PGPR and ascribes a new role for bacterial VOCs in triggering plant defense responses (Raupach and Kloepper 1998; Murphy et al. 2000).

An important factor of the competitiveness of PGPR is the ability of the bacterium to persist and proliferate. Under cold and temperate climates, many fungal phytopathogens are most destructive when the soil temperature is low. Hence, it is reasonable to expect that the use of PGPR that is cold tolerant will be much more

effective in the field than mesophilic BC strains. The ability of some PGPR to hydrolyze 1-aminocyclopropane carboxylate (ACC), the immediate precursor of ethylene in plants and a compound naturally found in root exudates, may provide these strains with a competitive advantage over other microorganisms in the rhizosphere because they can use ACC as a source of nitrogen (Glick et al. 2007).

In an effort to engineer a more soil-persistent BC bacterium, NAH7 plasmid which carries the gene encoding enzymes of the naphthalene and salicylate biodegradative pathway was transferred into an established BC strain (Doke 1983). Plant roots may also respond to colonization by PGPR by producing active oxygen species (Katsuwon and Anderson 1990; Glick and Bashan 1997). It should, therefore, be possible to manipulate genetic of PGPR, to increase the levels of one or more of the enzymes that reduce the number of active oxygen species so that PGPR strains with an increased root colonizing ability, and hence increased effectiveness against fungal pathogens might be created.

4.9   Conclusion

To achieve sustainable crop production to feed a growing global population, strategic measures should be taken on the management of the environmental problems such as abiotic and biotic stresses (phytopathogens and insect pests) as the major constraints to the food production worldwide which affects yield loss of the agricultural production.

One of the approaches/strategies to reduce the use of chemical fertilizers and pesticides in agricultural crop production has been done by large-scale application of PGPR as inoculants to increase crop yield as well as agricultural sustainability. In the process of healthy growth of plants, the PGPR strains made a significant contribution in different ways, whereby the PGPR was localized on the surface of plant roots and also can protect the plant from biotic stress.

The PGPR plays a very important role in helping the plant grow to adapt to the environment. They have essential functions in microbial antagonism, as well as are able to elicit induced resistance. Resistance-inducing and antagonistic rhizobacteria might be useful in formulating new inoculants, offering an attractive alternative of environmentally friendly BC of plant disease and improving the cropping systems into which it can be most profitably applied. These new PGPR will require a systematic strategy designed to fully utilize all these beneficial factors, applying combinations of different mechanisms of action allowing crop yields to be maintained or even increased while chemical treatments are reduced.

The PGPR strains can directly inhibit the pathogen by their antagonistic properties mostly for soilborne diseases, while the PGPR strains can induce systemic resistance and trigger ISR through JA/ETH and/or SA signaling pathways for mostly plant shoot/leaf disease. The application of some PGPR strains can induce systemic resistance to some agricultural pests and diseases, and the process mainly occurred by activating JA signaling pathways.

Laboratory study and field trials of PGPR have opened up a new era for the agricultural bioinoculant industry. Development of superior or novel PGPR strains with improved plant growth promotion traits and development of transgenic crop plants expressing PGPR gene with increased resistance to various

biotic stresses are possible through genetic manipulations. These PGPR technologies can be exploited as a low-input, sustainable, and environment-friendly technology particularly for the management of biotic stresses.

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